Research article

STUDY OF THE MICROBIOLOGICAL AND PHYSICOCHEMICAL QUALITY OF RAW MILK FROM COWS EXPOSED TO ENVIRONMENTAL POLLUTANTS IN THE REGION OF WEST ALGERIA

BENLAHCEN K., MOULOUDI F., KIHAL M.

Faculty of Sciences of Nature and Life (SNV) Department of Biology, Applied Microbiology Laboratory (AML) University of Oran 31000 Algeria

Author for correspondence: <u>benlahcen.k@gmail.com</u> Tel/fax: +213334850

Abstract

The environment is threatened constantly by noise and industrial pollutants including toxic fumes and heavy metals (arsenic, cadmium, lead and zinc etc...). In addition, high household hazardous wastes are dumped haphazardly in the open air [1, 2,3]. For lack of grass hay and water, it is in these places that some cows in Sidi Bachir on the outskirts of the Wilaya of Oran find their pasture. This research has two components: 1) Microbiological and physicochemical study of raw milk of cows feeding mainly on waste dumped in nature. 2) A Behavioral study that for ethical reasons the experiment is performed in a laboratory at the University of Oran, on thirty (30) Wistar rats. The Group A is subject to poisoning lead acetate (a heavy metal that can probably be found in the place of pasture) at a dose of 500 mg/l, and the Group T (control rats), which receives clean tap water. The results show neurological and behavioral disorders, which is in favour of the involvement of serotonergic transmission system. 3) In order to deepen the involvement of the different routes of transmission, we undertook a Biochemical study to reveal stress hormones: prolactin in female rats and corticosterone in male rats. The results show in the first place, evidence of microbial contamination of raw milk with high levels of impurities, whose presence is probably due to non-existent hygiene practices in the areas of housing of animals, grazing and milking. Second, chemical pollution is causing a decrease in nerve transmissions (catecholamine, glutamate and serotonin). Finally, hormonal dosage of corticosterone and prolactin showed that lead causes a decrease in fertility in addition to a state of anxiety and stress in animals. Copyright © IJESTR, all rights reserved.

Key words: Raw milk, Chemical pollution, Neurobiology of behaviour, Wistar rats, Hormone Stress.

Introduction

From the definition of the concept of environmental health proposed by WHO -the World Health Organization-(Helsinki, 1994), it is clear that the health of the fauna and flora is influenced strongly by the environment through the exterior items such as water, air, waste, noise and radioactivity [1]. Major algerian cities such as Oran (located 400 km to the west of Algiers) have became shanty-town, as a result of increased human activities, generating increasing amounts of physical, chemical and biological agents with a power of blistering toxicity and the potential risks to the well-being of the population.

In Algeria, the efforts of research in the field of pollution are mainly oriented toward the environment in general. However, the application side is still poorly controlled, particularly at the level of the struggle against exposure to chronic contamination by heavy metals, industrial smoke and household waste. Because of this, if we take the milk as essential food item to the power supply, it is important to know that there are a number of situations where the consumption of milk from cows, sheep or goat may be unfavourable and even dangerous for the health of man. Several authors [2,3,4] agree to say that according to the rearing conditions, in cattle, sheep and goats; the breasts are real emunctory. Raw milk may contain substances ingested or inhaled by the animal, either in the form of the original grantor, or a derivative compound metabolised. Milk provides a matrix easily accessible, rich in a wide variety of essential nutrients: minerals, vitamins and proteins easy to digest. Different foreign substances can come from the food (inappropriate food and water for livestock, inappropriate fertilizers and plant protection products), the environment (heavy metals, pesticides, radioactive elements), of prescribed treatment to the animal (pharmaceuticals, antibiotics and hormones). The heavy metals and their salts (zinc, copper, lead, tin, and mercury) are many and very toxic and traces of these heavy metals maybe found where the cows graze and drink. The focus of our attention is given to the insidious nature of lead and its effects on the nervous system. For example, in pregnant fauna, this metal, attaches itself to a protein, crosses the placental barrier and reaches the fetus. This hazardous metal can intoxicate the body as well as the milk of the animal [5]. To the sides of the chemical pollution, the noise may constitute a noise pollution permanently affecting the environment and homeostasis in the individual and inducing neurological disturbances [6, 7, 8,9] that can lead to hearing loss. The quality of the milk of mammals provides information on the status of the health of the animal, its food, its mode of life and its habitat [9, 10, 11,12]. The pathogenic micro-organisms can enter the body of the man or animal through the power supply, breathing, dermal absorption and the daily exposure to the countless chemicals created and employed by man (10,11.13,14]. In effect, when the individual is located in a polluted environment, it shows signs of stress with fatigue, pallor, loss of appetite (which may sometimes lead to anorexia), irritability, sleep disturbances, anxiety, constipation, headache, taste and behavioral disorders [15, 16, 17,18]. Our study focuses on two strands: a) a microbiological study and physicochemical study of the milk of cows living on the outskirts of Oran in western Algeria. b) a behavioral study and neurobiochemical study and for reasons of ethics we take the rat Wistar as biological material.

Materials and Method

A/ The microbiological surveyof cow's milk

1. Sample and sampling

We are conducting a microbiological and physicochemical study of raw milk from cows collected from four sites all located in the region of Oran. The cows of site 1 are nourished daily in an illegal improvised public dump bordering Sidi Bachir. The site 2 is located in the commune of Messerghine where the rearing conditions of the cows are acceptable. The site 3 is located in the locality of Fleurus where the cows rearing conditions are very modern and hygienic. Finally, the site 4 is located at Gdyel, where the cows roam freely in semi hygienic conditions. The milk of six cows from each site is mixed and analysed.

2. Physicochemical Analysis

2.1 Measurement of pH:

On the arrival of the samples of raw milk to the laboratory, the pH is measured using a pH-meter. After calibration the pH 7.02 and 4.00. The pH measurement is made by immersing the end of the electrode in the curdled milk.

2.2 Measurement of the Dornic acidity milk hydrometer with thermometer:

To a volume of 10 ml of raw milk, 2 or 3 drops of phenolphthalein is added to a beaker. After homogenisation of the mixture, the washing soda is added dropwise in to the buret, until the solution turns from colorless to pink. This coloration must persist for at least 8 seconds to pH 8.4 The result may be expressed in degrees Dornic milk hydrometer with thermometer ($^{\circ}$ d) or in gram of lactic acid per liter of milk (g/l).

2.3 Measurement of the density:

The density is measured using a thermo-lactodensimeter. It is reduced to 20 °C by the formula: corrected density = density read +0.2 (milk temperature 20 °C

2.4 Determination of the extract total:

Total dry matter is done by drying in the oven set at 103 ± 2 °C. After an evaporation of the water by means of water bath boiling point, density is determined using a lactodensimeter.

3. Microbiological Analyzes

In the raw milk, one can estimate the pollution and to enumerate several types of pathogenic bacteria, such as : the *Staphylococcus aureus*, and the *Staphylococcus faecal*; their pathogenicity may cause of the toxico-food infections or TIA [19.20].

3.1 Enumeration of the total flora (FMAT) :

The purpose of this manipulation is to count all the microorganisms present. In order to estimate the pollution and the general microbiological quality of the product at 30°C (for duration of 24 hours).

-We carried out a series of dilutions aseptically $(10^{-1}, 10^{-2} \ 10^{-3})$, we take 1ml of each dilution in an empty Petri dish. We added 15 ml of melted agar PCA (Plate Count Agar) and then cooled it to 45 ±1 °C. We proceed to homogenise the inoculum with the agar per circular movements to and fro. We leave it to solidify and then incubate it at 30 °C for 72 h.

-The enumeration of Petri dishes containing microorganisms to the dilution of 10^{-3} , is done on the observance of the standard set by the legislation [21]. The seeds appear in the form of colonies different in size and shape.

3.2 Research of Staphylococcus aureus

After melting, the middle of Baird Parker solid is placed in Petri dishes. We spread 0.1 ml of the inoculum diluted, on the entire surface area of the box. The incubation period is 24 to 48 hours at 37 °C. The colonies of Staphylococcus aureus appear, black, shiny, curved, and surrounded by a clear halo of approximately 2 to 5 mm in diameter.

3.3 Research of *fecal Staphylococcus:*

We introduced aseptically 1 ml of raw milk non-diluted in five tubes of middle of Rothe, while achieving the dilutions at 10^{-1} , 10^{-2} , and 10^{-3} . In all five tubes, we added 1 ml of each dilution. The incubation is carried out in an oven at 37 °C degrees celsius for 48 hours. If the contents of the tubes are positive (that is to say cloudy), we then go on to introduce a little of that positive content to the middle of Litsky. (With a cove of platinum and submitted to an incubation at 37 °C for 48 hours. In the case of a homogeneous cloudy occurrence and a violet disc at the bottom of the tubes, indicating that there is the presence of *faecal streptococci*.

Expression of results:

To determine the estimated total number of aerobic flora mesophilic in a gram of food. We must therefore retain the counting of boxes containing between 30 and 300 colonies, obtained. For each micro-organism characteristic, the number of micro-organisms per gram of sample is equal to:

$$N = \frac{\Sigma C}{(n1 + 0.1 n2) d1}$$

N: Number of CFU per g or per ml of initial product; Σ C: sums of the colonies of interpretable boxes;

V: Volume of solution filled (ml);

N1: Number of boxes considered in the first dilution restraint;

N2: Number of boxes considered in the second dilution restraint;

 \mathbf{D}_1 : Factor of the first dilution restraint.

B/ The behavioral study in Wistar rats

The purpose of this study is to simulate the exposure of a human or any other animal to chemical spills and to noise pollution. We proceeded with the macroscopic observation with the naked eye of the behaviour of treated rats (intoxication with heavy metals) according to the method of Irwin (1968). For ethical reasons, we have opted to support the microbiological and physicochemical results via experimentation on Wistar laboratory rats. The concepts and methods of the biology of behaviour would help to confirm some experimental data as is presented in biological sciences (particularly in neuropsychology).

1 /Sampling

The animal model chosen for our experiment is the white rat, species *Rattus norvegicus*, strain *Wistar*. All the rats are free of specific pathogens (IOPS), or (SPF: Specific pathogen-free). They are placed in collective cages (with feeding bottle), they are grouped by batch of 5 animals, kept in a controlled environment characterized by a cycle of artificial light (12h/12h, day/night), a relative humidity of $40 \pm 5\%$ and an ambient temperature of $22 \pm 2^{\circ}$ C.

1.1. Group A consignment has a number of 10 Wistar rats (five male and five female), whose daily intake of drinking water is diluted with lead acetate ($C_4H_6Pb_2H_2O$).

1.2. The GroupT is composed of ten wistar rats (five male and five female) they represents the control animals receiving tap-water and are placed in a quiet room. The average age of all rats is 23 ± 2 days (after weaning) and the average body weight of 46 ± 2 .

2. The experimental protocol according to Irwin [22]

The technique of Irwin is a macroscopic study involving an observation phase and a phase of handling of the rats (3 to 5 minutes per subject). The criteria is rated according to a scale agreed, this allows you to compare between the effects in a satisfactory manner.

A set of thirteen stereotypical behaviours are studied: the body posture, the locomotor activity, the earthquake, the spontaneous outburst, the leap to the touch, the grasping reflex, the reflex action of reorganization, the abdominal tonus, watery eyes, the pinch of the tail, the mortality, the cry and finally direct observation.

Depending on the intensity and the degree of the behavioral effect, we allocate the signs $(-, +, \pm, ++, +++)$.

For each Wistar rat (treated or control animal), an individual sheet is established. The normal score is logged in column **CN** (*normal behavior*), in column **CA** (*abnormal behavior*) and in column **CA** *(*intense abnormal behavior*). During the course of the tests, the presence and intervention of the experimenter are limited.

The exploration of statistical results is carried out using the test 'T' of Student-Fisher .

P < 0.05 is regarded as significant, P< 0.001 and is considered as very significant.

3. Dosages of hormones markers of stress

At the end of 04 weeks of experimentation

The animals are sacrificed after a 12 hour fast using an intraperitonial injection of a solution of chloral hydrate $(C_2H_3Cl_3O_2)$ to 10% (4mg/kg body weight). After incision of the abdomen, the viscera is discarded in order to collect the blood through a puncture of the abdominal aorta. The blood is placed in heparinised tubes and then centrifuged at 3000 revolutions/10 minutes. The plasma is collected and then stored at 4C° for hormonal dose markers of stress (corticosterone, prolactin) by the use of an AbottAxSYM Kit.

3.1 Doses of the Corticosterone: dosing is carried out in male control rats (Group T) and intoxicated male rats (Group A).

3.2 Doses of prolactin: dosing is carried out in female control rats (Group T) and intoxicated female rats (Group A).

Results and Discussion

The results of the physicochemical and microbiological analysis from the samples of the four sites confirm the close relationship that links the quality of milk and the conditions of farming to the standards of hygiene within the environment in which the herd is reared. The contamination of dung, by micro-organisms is an indicator of potential risk of contamination of the milk. The physicochemical analysis data is illustrated in **Table I**.

The acidity of the milk is a good indicator of its quality and its freshness at the time of consumption or of its delivery. The acidity allows you to assess the amount of acid produced by the bacteria and detect possible fraud.

Indeed, the normal fresh milk must be at neutral pH and the samples of raw milk collected from the four sites tested show a variation with regard to their hygienic microbiological quality.

The degree of Dornic milk is measured by hydrometer with thermometer and is in the range of 17 to 20 ; the differences between the values is not important. However, the results of the average density of the various samples collected are between 1029 and 1032 inclusive. The decrease in the density of samples collected at Sidi Bachir would probably be due to the cow fodder (poor nutrition). In effect, the density depends on the content of dry matter, fat content, the temperature increase and especially on the availability of food (Table I). The results from **site 1** show that the total flora is 4. 10^5 , Which is within the standards reported in the Official Journal of the Algerian Republic N°35.

Samples	РН	°DORNIC	Density	MG/gl	IS g/l
Sidi Bachir	6.3	20	1029	31.3	118
Messerghine	6.6	17	1030	28.3	96
Fleurus	6.70	18	1032	22.6	92
Gdyel	6.59	20	1031	24.5	100

Table 1: Results of physicochemical analysis of 4 sites

MG/gl : butterfat in gram/liter, IS g/l: dry extract in g/l

The results reported in the previous table (Table I) reveal the presence of significant variations between the various sites studied, including the values of the fat content (MG) and in total dry extract (IS). Our results give us the values for a fat (MG) oscillating between 28.3. Each g/l and 31.3 g/l and between 92g/ and 130g/l for the dry milk extract (IS). According to Gonzalo in 2005 [23], reported that in cold season the water content of milk decreases and therefore its dry matter increases . The climate and the nutrional supply are important factors that affect the variation between the composition of the milk fat content as well as the dry milk extracts.

Results of microbiological analysis : The enumeration of the aerobic flora mesophilic total (FMAT), of *faecal streptococci* and *Staphylococcus aureus* gave the results shown by the **Table II.**

Knowing that in the conditions of normal hygiene (where milk produced is considered fit for human consumption), the flora mesophilic aerobic total (FMAT) rotates around the 25000/g to 50000/g. Thence if the (FMAT) is more than 50000/g the product is considered to be unfit for human consumption. We observed that the raw milk from site 1 Sidi Bachir, had a rate of 86% of contamination ; which does not meet the standards recommended by the Algerian legislation [21]. This result is a sign of poor hygiene and habitat conditions of animals. Indeed, the different milk analyzed show a flora greater than $10,10^5$; compared to the results found in the USA (24) and in Brittany [25] or 5% and 2% respectively of the samples contained a flora greater than $10,10^5$. On the contrary, the site 3 Fleurus which shows signs of very good hygiene conditions with a very low rate of contamination around the zero. Animals seem in perfect homeostasis with their environment. The sites 2 (Messerghine) and 4 (Gdyel) with 34% and 11% respectively, have little less of contaminations but still disturbing.

Table II: summary results of the Microbiological analysis and frequencies of microbial contamination of samples in the 4 sites

Place	FMAT (CFU/ml)	CF (CFU/ml)	SF (CFU/ml)	Staph aureus.Aureus (CFU/ml)	Percentage of contamination
Sidi Bachir	72,3.10 ⁵	8,4.10 ⁵	9,8.10 ⁴	19,4.10 ⁵	86%
Messerghine	$38,10^4$	<10	ABS	ABS	34%
Fleurus	$2,1.10^2$	ABS	ABS	ABS	0.92 %
Gdyel	$27,1.10^5$	3,6.10 ⁴	$2,7.10^4$	ABS	11.4 %

The contamination by the FAMT is very important because 81.2 per cent of milk analyzed show a flora greater than 10^5 cfu /ml. This situation is very worrying compared to that reported by other researchers [24,25], which saw that the microbiological quality of the milk is important for its conservation and even its transformation and its consumption.

In the site 3 (Fleurus), there is a lack of contamination by faecal coliforms and faecal streptococci, this may be due to the fact that the site is modern. Stables with milking mechanisation where the washing of the teats are systematic. The use of a metal teatwasher means lower rates of faecal coliforms. The higher rates coliforms are proof of unsanitary conditions during or after the processing of the product. In site 1, site 3, and site 4, the milking is done manually, which increased the risk of exogenous milk contamination of faecal origin by increasing the contact surface between the milk and the micro-organisms in the environment, especially when that this last is defiled.

It is to be noted that the strong contamination observed particularly in the site of Sidi Bachir easily explained and confirmed the total neglect of the breeder as to the unsanitary conditions of the habitat of animals and the poor conditions of milking. Note that the raw milk collected from a healthy cow, contains a small quantity of microorganisms and its protection against the bacteria is ensured by inhibitory substances but only for very short duration. Our results are in agreement with those found by other authors [26, 27, 28].

The faecal contamination allows you to judge the hygienic condition of samples of milk. It is true that the coliforms do not persist long in the food. But their disappearance does not mean that the risk of finding a pathogen has disappeared. The presence of *faecal streptococci* in the samples of Sidi Bachir and Messerghine, considerably significant in

The presence of *faecal streptococci* in the samples of Sidi Bachir and Messerghine, considerably significant in comparison to sites of Fleurus and Gdyel (Table II).

These pathogenic organisms constitute a real risk to public health in the processed products as it can produce, in certain conditions, thermostable enterotoxins which can withstand heat treatments. The presence of pathogenic microorganisms in the milk at the considerable rate of 86% seems to be reflective of the poor hygienic conditions of the farms in the region of Oran. Several work [29, 30, 31), including those of Baazize in 2005, have reported presence of pathogenic microorganisms at a rate of 91.09%. Our study gives a rate approaching that in all samples (except site 3 Fleurus). This qualifies as very bad because the rates of pathogenic microorganisms far exceed the standard recommended by the official journal [21] concerning the microbiological criteria of milk and milk products.

Overall the presence of this flora diversity, either fecal or pathogenic is as a direct consequence of lack of basic good practices between farmers and the veterinarians(the non- compliance and the ignorance of rearing conditions.... the total lack of hygiene measures, in particular those relating to the cleanliness of animals and their housing.)

According to the FAO in 1998 [33], the contamination of milk by this bacteria can cause major problems in public health and cause food poisoning, as they can affect the conservation and the transformation of the raw milk. Our result reveal the presence of faecal contamination observed with high rates in the soiled milk or their presence is due to the practices of

inadequate hygiene for rearing, breeding and milking. Moreover the presence of germs regarded as pathogens such as *Staphylococcus aureus* and *Escherichia coli* also reflects the health status of the animal. That is that the cows live in unhealthy and unhyigenic conditions. With regard to site 3 Fleurus, it reflects a good condition for breeding, hygiene and that the animals there live in a healthy environment managed by a qualified staff.

Results of the behavioral study :

Sometime because lack of water some breeders store water in metal or dug up reservoirs. This water is of poor quality and is contaminated with microorganisms and heavy metals. This water is used to water the animals and the contaminted content thats in the water is also ingested through the whole body of the drinking animal. The presence of lead or any other heavy metal in the water supply of the animal is a source which not only affects the central nervous system (CNS), but also the behavior of stereotypical movements that can cause the cannibalism in Wistar rat, as well as depression. The summary Table III gives the results of some stereotyped behaviors in Wistar rats submitted to chemical pollution.

Lots of animals	Group T	Group A
S Y M P T O M A T O L O G I E	_	
Body Posture	++	±
Locomotor Activity	++	±
Shakiness		++
Spontaneous Burst	-	++
Jump to the touch	+	±
Reflex give you better grasping ability	++	±
Reflex of recovery		+
Abdominal Tonus	+	±
Lachrymation		±
Toe-in of the tail	++	±
Mortality	±	+++
Cree	±	++
Comment	CN	CA

Table III: Summary Results of the Test of Irwin simplifies

Group T: rats witnesses non-poisoned and living in a quiet environment Group P : rats poisoned 500mg/l of lead acetate dissolved in the drinking

-- observed effect null ; ±moderate effect ;++ net effect ; +++ Effect intense ; CN: normal behavior ; CA: abnormal behavior intense

Effect of chemical pollution on the health of rats

Certain behaviours such as the shakiness, the spontaneous bursts, the instinct for reorganisation and cry are more marked in rats with lead intoxication than in non toxicated control rats. This could be the result of a neurological attack causing abnormal behavioural disorders in the intoxicated rat in comparison to the control rat. It is to be noted

that some animals die before the end of the experiment. Figure1 shows a rat when stressed, shrivels up in the bottom of his cage during chronic exposure to lead acetate at a dose of 500 mg/l for a duration of four weeks. However, Figure 2 shows the image of a very relaxed rat, strolling and exploring his new environment. At the end of the experiment, we observe that the neurological disorders are significant among Group A in comparison with the control (Group T). The rats poisoned have fear and seek to flee. They are very sensitive to the pinching of the tail and flinch at the touch.

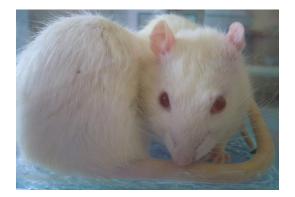


Figure 1 : Rats in a corner of his cage, after 4 weeks Of chronic intoxication with lead acetate. Stressed and shows signs of neurological disorders, the rats shrivels themselves up



Figure 2 : Rat witness very relaxed at the level of the center of a labyrinth exploring his new environment.

The rate of prolactin and Testosterone : In control rats, the rate of dosage of prolactin blood has revealed that, the prolactinemie had the value of 183.2 ± 4 ng/ml. However, among the addicted to the lead acetate, the rate of prolactin increased to 258.25 ± 5 ng/ml (Figure 3).

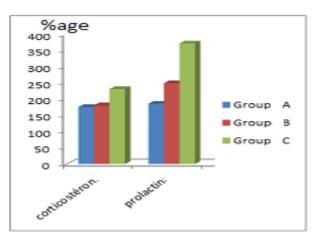


Figure 3 : Results of stress hormone doses (Prolactin and corticosteron)

The rate prolactin significantly increases attesting that the behaviour is of pituitary origin involving the mechanism of feed- back. In addition to the emergence of factors activating the release of prolactin (PAF). The heavy metals disrupt not only the mechanisms of neurotransmitter release regulated by the calcium, but also leads to damage at the level of the mechanisms of DNA repair. Its role of inhibitor could originate from its interaction with the sites likely to be occupied by the calcium and a liberation too important or too prolonged of glutamate caused a neuronal death [35]. It has been observed in a previous experiment [36] that the lead is on the tracks of the nerve transmission in rats or they are completely collapsed on the back legs. The lead would also lead to a reduction in the

number of new born and in their birth weight markedly of 17 %. Similar work have shown that the lead causes of premature births in pregnant female and a corruption of the growth and fetal development [36, 37,38].

Several studies including those of Wearden and Appleby in 1997 [39] have demonstrated that lead exposure pre and post-natal, even at low levels, has an influence on the cognitive performance and behaviour of the child. There would also be a disturbance of the growth. Pregnancy and Breastfeeding promote the cytokine release in the circulation of lead stored in the bones of the mother; the lead passes the placenta barrier; the absorption of lead by the mother is increased during gestation (up to70% of the lead ingested instead of 20% outside of the pregnancy); the lead passes into breast milk; the central nervous system (CNS) of the fetus and the newborn is in full development. Heavy metals induce a chronic stress resulting in an alteration of some receivers to serotonin (increase of the receptors 5-HT2a and decrease of receptors 5-HT1a). The lack of serotonin can have harmful effects on the neurological status and of this fact, it promotes the emergence of stress. To this effect the lead is an inhibitor of serotonin reuptake, that is to say, it prevents its recapture by the nerve fiber presynoptique thereby increasing its concentration in the synapse and strengthening its effect at the level of the fiber post synaptic. The stress exhaust serotonin, therefore, it becomes more difficult to manage in the absence of this neurotransmitter [37, 38,39]. Once the animal is under stress, the effect of the heavy metal is manifested *in utero* or at the time of parturition (calving of animals). Sometimes, abortion in animals is due to bacteria passing in the amniotic fluid, and swallowed by the fetus, causing a fatal septicaemia. In general an infected female does not abort, only once (in 80% of cases), but it remains infected and may excrete the bacteria in the milk and genital secretions during the following calving [39]. The cows in loose housing or grazing in a contaminated environment can be infected in contrast to those in a stabling

of type attached. The transmission is by direct contact with manure or decomposed toxic waste of the public dump in the case of Sidi Bachir. Indeed, in the case of lesions, the spontaneous cure would be compromised as well as the microbiological quality and physicochemical of milk from these animals [40, 41].

Conclusion

The high rates of contamination observed (staphylococcus aureus bacteria faecal) found in the soiled milk of Sidi Bachir, testify to a poor awareness of the breeder as to the health of the animals and therefore the health of the consumer. The presence of pathogenic germs in milk is due to inadequate hygiene practices during grazing, stabling, milking and the packaging of milk and its transport to the consumer. In the final analysis, it is evident from this study that the raw milk undergoes severe contamination and the failure of high hygiene standards of the farming materials and the multiple manipulations constitute this origin.

The first emergency is to prepare an inventory of the risk of contamination of milk, to define a coherent plan of action, to discuss the priorities and to be aware of the responsibility of the local actors as the APC (Popular Assembly communal) and the Wilaya (prefecture), need to face the contamination and the degradation of the environment. The ideal would be to apply the laws relating to the environment and get away from the unsuccessful rhetoric, while assessing the complex role that information can play on the populations awareness of environmental degradation. Overall, our results lead to the need to establish a policy of quality among the breeders by the veterinarians, and insist on the cleanliness of the animals and their environment.

Perspective

It is time to take conscience in order to preserve the environment and to consider:

* how do we get to better assess and prevent the risk of pollution and how can it be remedied?

* What are the key ways of contributing to a better fundamental understanding on contaminants in order to identify the appropriate preventive measures, and reduce their potentially harmful effects on ecosystems, human health, animal and plant?

Acknowledgement

We thank Miss M. M. E. A. E. Khoder and Miss Sadeki I.F. for the translation of this article.

References

[1] Rychen G., Ducoulombier-Crepineau C., Grova N., Jurjanz S., Feidt C.(2005), Modalités et risques de transfert des polluants organiques persistants vers le lait, INRA Productions Animales, 18, (5), 355-366.

[2] Pagliuca G., Serraino A., Gazzotti T., Zironi E., Borsari A.,Rosmini R.(2006), Organophosphorus pesticides residues in Italianrawmilk, J. DairyRes., 73, (3), 340-344.

[3] Menard.C, Heraud.F, Nougadere.A, Volatier.J, Leblanc.J-C, (2008), Relevance of integrating agricultural practices in pesticide dietary intake indicator, Food and Chemical Toxicology. 46, 3240-3253.

[4] Sattler.C., Kachele.H., Verch.G. (2007), Assessing the intensity of pesticide use in agriculture, Agriculture Ecosystems & Environment 119: 299-304.

[5] JORA Décret exécutif n°93-184 du 27 juillet 1993 réglementant l'émission des bruits.
[6] Aloisi, A.M., I. Ceccarelli and C. Lupo, 1998: Behavioural and hormonal effects of restraint stress and formalin test in male and female rats. *Brain Research Bulletin* 47(1): 57-62. ISSN: 0361-9230.

[7] Sida, P., M. Koupilova, S. Hynie, and V. Klenerova (2003). Effects of two types of restraint stress on the learned behaviour in rats. *ActaMedica Hradec Kralove Universitas Carolina, Facultas Medica Hradec Kralove* 46(4): 153-6. ISSN: 1211-4286.

[8] Yoon, H., W.S. Chung, Y.Y. Park, and I.H. Cho (2005). Effects of stress on female rat sexual function. *International Journal of Impotence Research* 17(1): 33-8. ISSN: 0955-9930.

[9] Yoon, H., W.S. Chung, Y.Y. Park, and I.H. Cho (2005). Effects of stress on female rat sexual function. *International Journal of Impotence Research* 17(1): 33-8. ISSN: 0955-9930.

[10] OMS : Organisation Mondiale de la Santé (1989). Lead-environmental aspect. Genève, Organisation Mondiale de la Santé, *Critères d'hygiène de l'environnement*, N°85.

[11] Benhamed N., Moulay M., Aggad H. and Kihal M., 2011 : Prevalence of Mastitis Infection and Identification of Causing Bacteria in Cattle in the Oran Region West Algeria. *Journal of animal and veterinary Advances* 10 (22):3002-3005.

[12] Roudaut, H. Lefrancq, Bordeaux E., 2005 : Microbiologie et toxicologie des aliments - Hygiène et sécurité. *Edition INRS. Parution : octobre 2008*.

[13] Courtet Leymarios F., 2010 : Qualité nutritionnelle du lait de vache et de ses acides gras. Voies d'amélioration par l'alimentation. Thèse de docteur vétérinaire ; Faculté de médecine de Creteil France. 128 pages.

[14] Arakawa, H. 2005: Age dependent effects of space limitation and social tension on open-field behavior in male rats. *Physiology and Behavior* 84(3): 429-436. ISSN: 0031-9384

[15] Thoreux-Mabnlay Veleszdela A., Calle JF, Olivier MF., Soufir JC, Masse R. and Pinon- Lataillade G., 1995: Impairment of testicular endocrine function after lead intoxication in the adultrat. Toxicology. 26; 100(1-3);101-9.

[16] Alexander BH., 1996: Paternal Occupational lead exposure and pregnancy outcome. Int.J. Occup. environ. heath, 2(4). p280-285.

[17] Murray A.M , Wad dington J.L ., 1989: The induction of proming and vacus chewing by a series of selective D1 dopamine receptors , Agonists two directions of D1 ,D2 interaction, Eur. J. Pharmacol.,160 , 377-384].

[18] Winneke G., Kramer U., 1997. Neurobehavioral aspects of lead neurotoxicity in children. *Central European Journal of Public Health* 5: 65-69.

[19] Bourgeois C.M., Larpent J.-P.,1996 : Microbiologie alimentaire: Aspect microbiologique de la sécurité et de la qualité des aliments. Tome 1. 2e Ed. Tec & Doc., 704p.

[20] Murat M., 2009 : Nutrition humaine et sécurité alimentaire. Tec. & Doc. Lavoisier Paris. P678.
 [21] JORA : Journal officiel de la République Algérienne Démocratique et Populaire. Loi 038 du 03-06-1998.
 Décret exécutif n° 98-188.

[22] Irwin S., Comprehensive observational assessment, A systematic, quantitative procedure for assessing the behavioural and physiologic state of the mouse, Psychopharmacologia (Berl.) 13 (3), 1968, p.222-257.

[23] Gonzalo C., Blanco A., Beneitez E., Juarez M T., Martinez, Linage B., Ariznabarreta A. 2005, : Qualité physico-chimique et hygiénique du lait de brebis chez les troupeaux du bassin de castilla-Léon (Espagne). Rens. Rech. Ruminants, 12, 401.

[24] Boor K.J.,Brown D.P., Murphy S.C., Koslowski S.M., Bandlar D.K. :1998, Microbiological and chemical band quality of raw milk in new York state. J.Dairy Sci.81,1743-1748.

[25] Guinot Thomas P., Ammoury M., Laurent F. 1995: Effects of storage condition on the composition raw milk. *Int. Dairy J.*, 5,211-223. 15

[26] Bettache Guessas, Miloud Hadadji, Nouredine Saidi, Mabrouk Kihal, 2007 : Inhibition of Staphylococcus aureus growth by lactic acid bacteria in milk. *Africancrop Science Conference Proceeding vol. 8, p: 1159-1163*

[27] Kashifa K., Ashfaque M., Hussain I., 2001 : Bacteriological studies on raw milk supplied to Faisalabad city during summer months, Pakistan. Vet. 21, 2,77-80.

[28] Kihal M., Henni DE., Prévost H. et Diviès C. (2006) A new manometric method for measuring carbon dioxide production by dairy starter cultures, a case of *Leuconostoc mesenteroides*. African Journal of Biotechnology, 5, (4): 378-383

[29] Mohamed Salih Barka, Mebrouk Kihal, 2010 : Prevalence of Escherichia Coli Enterohemorragic O157:h7 in Frozen Bovine Meat in Algeria. *Journal of Applied Sciences Research*, 6(11): 1576-1580© 2010.

[30] Aggad. H, Mahouz. F., Ahmed Ammar.Y., Kihal.M. ,2009 : Evaluation de la qualité hygiénique du lait dans l'ouest algérien *Revue Méd. Vét.*, 160, 12, 590-595

[31] Ashnafi M., 1996, Effect of container smoking and incubation temperature on the microbiological and ergo a traditional Ethiopian sourmilk. *International Dairy J.*, 6 pp. 94.

[32] Baazize D., 2005 : Qualité hygiénique et sanitaire du lait cru de vache. Mémoire de Magistère en hygiène et qualité du lait, Université Saad Dahleb de Blida (Algérie).

[33] F.A.O. Organisation des Nations Unies pour l'alimentation et l'agriculture, 1998 : Le lait et les produits laitiers dans la nutrition humaine, Collection FAO: Alimentation et nutrition, n° 28, ISBN 92-5-20534-6

[34] Gulson BL et al., Relation ship of lead in breast milk to lead in blood, urine and diet of the infant and mother, Environmental Health Perspectives, 1998 ; 106(10) : 667-674 (BI 138 308).

[35] Botez Banks EC., Ferriti LE., Shucard DW., : Effect of low level lead esposure on cognitive function in children ; *review of behavioral, neurophysiological and biological evidence*, Neurotoxicology., 18, 1997, p. 237-281.

[36] Aloisi, A.M., I.Ceccarelli, Lupo C., (1998): Behavioural and hormonal effects of restraint stress and formalin test in male and female rats.*BrainResearch Bulletin* 47(1): 57-62. ISSN: 0361-9230.

[37] Benlahcen K. and Slimani M., 2007: The effect of the poisoning by the safe lead on the behaviour of the wistar rat, *Egyp J. ApplSc.22 : 398-405*.

[38 Marshall PS, Forstot M, Callies A, Peterson PK, Schenck Ch., 1997: Cognitive slowing and working memory diffulties in chronic fatigue syndrome. *Psychosom. Med.*; <u>59</u>: 58-66.

[39] Wearden A, Appleby L., 1997: Cognitive performance and complaint of cognitive impairment in chronic fatigue syndrome (CFS) *Psychol. Med.* ;27 : 81-90.

[40] Mami Anas, Hamedi Amine Rizk, Henni JamalEddine ,Kerfouf Ahmed& Kihal Mebrouk, 2010 : Activité Anti-Bactérienne de *Lactobacillus plantarum*isolée du lait cru de chèvre d'Algérie vis à vis de *Staphylococcus aureus*. Les technologies de laboratoire - Volume 5, N°21

[41] Srairi M. T., Hamama A., 2006 : Qualité globale du lait cru de vache au Maroc, concepts, état des lieux et perspectives d'amélioration. *Transfert de technologie en agriculture*, 1-4.