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INCIDENCE OF *TRUEPERELLA PYOGENES* IN RAW MILK

NAGAH M. SAAD; SAHAR MAHMOUD KAMAL AND
AMIRA GAMAL ABO-SAGHER

Dept. of Food Hygiene, Faculty of Vet. Med., Assiut University.

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ABSTACT

One hundred and eighty raw milk samples were collected randomly from dairy farms, farmers` houses and dairy shops in Assiut city. These samples represented by 60 milk samples from cows and buffaloes of dairy farms (30 for each) , 90 samples from farmers` houses including cows, buffaloes and sheep milk (30 for each) and 30 raw dairy shops` milk samples. The samples were examined for the incidence of *T.pyogenes* and prevalence of Subclinical Mastitis (SCM) in raw milk. The prevalence of SCM in milk samples of dairy farms were 90% for both cows and buffaloes` milk based on California Mastitis Test (CMT) and 50% and 46.7% in cows and buffaloes` milk based on White Side Test (WST), respectivey. In the contrary, prevalence of SCM in farmers` houses milk samples based on CMT and WST were 53.3%, 73.3% &33.3% and 33.33%, 46.7% & 50% in cow, buffalo and sheep, respectively. The incidences of *T.pyogenes* in dairy farms milk samples were 60% in both cows and buffaloes` milk, while 63.33%, 60% and 36.67% in cows, buffaloes and sheeps` milk of farmers` houses, respectively. Additionally, the incidence of *T.pyogenes* in dairy shops` raw milk samples was 43.3%. PCR for 18 strains of *T.pyogenes* revealed that 15 strains gave positive results for pyolysin virulence gene. The public health hazards of the organism and the suggestive measures were also discussed.

Key words: *T.pyogenes; Subclinical mastitis; California mastitis test; White side test*

INTRODUCTION

T. pyogenes is a worldwide known bacterium of genus *Trueperella* belonging to the family *Actinomycetaceae* in the order *Actinomycetales* of the class *Actinobacteria*. Genus *Trueperella* was named after Hans Georg Trüper, a German microbiologist (Yassin *et al.,* 2011). It is a Gram-positive, pleomorphic, non-spore-forming, non-motile, non-capsulated, facultatively anaerobic rod, which is characterized by a fermentative Metabolism and strong proteolytic activity (Abdallah, 2016). Growth of the bacteria occurs on ordinary media but is enhanced on media containing blood or serum. Colonies of *T. pyogenes* on sheep blood agar were described as pinpoint, convex, slightly translucent and circular and were surrounded by a zone of β-hemolysis (Schaal, 1986; Lämmler and Hartwigk, 1995). *T. pyogenes* is considered to be a part of the biota of skin and mucous membranes of the gastrointestinal, upper respiratory and urogenital tracts of animals (Rzewuska *et al.,* 2006; Silva *et al.,* 2008). Moreover, this bacterium was also isolated from the udders of clinically healthy

Corresponding author: Amira Gamal Abo-sagher
E-mail address: amira.gamal.abosgher@gmail.com
Present address: Dept. of Food Hygiene, Faculty of Vet. Med., Assiut Univ.

cows (Spittel and Hoedemaker, 2012). This opportunistic bacterium causes mastitis which is associated with a wide range of physical, chemical and microbiological changes of milk. (Radiostasis *et al.*, 2007) These changes are accompanied with clots, discoloration of milk and high number of leukocytes in the affected milk and it contributes to remarkable reduction of milk production, lower milk quality, affect fertility of cattle,in addition to high costs of control and treatment. In human, the infections are connected with occupational exposure (Kavitha *et al.*, 2010), including endocarditis (Jooter *et al.*, 1978), pneumonia (Amejeiras *et al.*, 2004), arthritis (Nicholson *et al.*, 1998), various purulent lesions and abcesses (Gahrn-Hansen and Frederiksen.1992) and sepsis (Levy *et al.*, 2009). *T.pyogenes* is armed with known numerous virulence markers, contribute to increase its pathogenic potential (Cohen *et al.*, 2015)

MATERIALS AND METHODES

Milk samples:

A total number of 180 random milk samples were collected from dairy farms, farmers` houses and dairy shops in Assuit city. Collected samples were transferred to laboratory as soon as possible to be examined for the incidence of *T.pyogenes* and presence of SCM. Storch test is applied on the samples to exclude the heat treated samples according to Lampert (1975).

1- Preparation of milk samples according to A.P.H.A. (1992)

2-Screening test to detect SCM for dairy farms and farmers` houses milk samples:

A- CMT according to Schalm *et al.* (1971).

B- WST according to Kahir *et al.* (2008).

3-Isolation and identification of *T.pyogenes* in raw milk samples (Quinn *et al.*, 2011):

4-Detection of virulence factor (pyolysin gene) of *T.pyogenes* using PCR (Cohen *et al.*, 2018): Oligonucleotide primer sequence:

Target gene	Primer sequence (5`-3`)	Length of amplified product	Reference
Pyolysin	GGC CCG AAT GTC ACC GC	270 bp	Cohen et al. (2018)

RESULTS

Table 1: Prevalence of SCM in the examined raw milk samples based on CMT and WST:

Type of milk samples	Positive milk samples for CMT		Positive milk samples for WST	
	No/30	%	No/30	%
Dairyfarm:				
-Cow	27	90	15	50
-Buffalo	27	90	14	46.67
Farmers` houses:				
-Cow	16	53.33	13	43.33
-Buffalo	22	73.33	14	46.67
-Sheep	10	33.33	15	50

Table 2: Incidence of *T.pyogenes* in the examined raw milk samples.

Type of the examined milk samples	No. of the examined samples	Positive samples	
		No.	%
Dairy farms:			
-Cow	30	18	60
-Buffalo	30	18	60
Farmers` houses:			
-Cow	30	19	63.33
-Buffalo	30	18	60
-Sheep	30	11	36.67
Dairy shops milk samples	30	13	43

Table 3: Correlation between the positive CMT, WST and incidence of *T.pyogenes* in the examined raw milk samples.

Type of the examined milk samples	No. of the examined samples	No. of positive CMT samples	No. of positive WST samples	No. of positive samples for *T.pyogenes*
Dairy farms				
-Cow	30	27	15	18
-Buffalo	30	27	14	18
Farmers` houses				
-Cow	30	17	13	19
-Buffalo	30	22	14	18
-Sheep	30	10	15	11

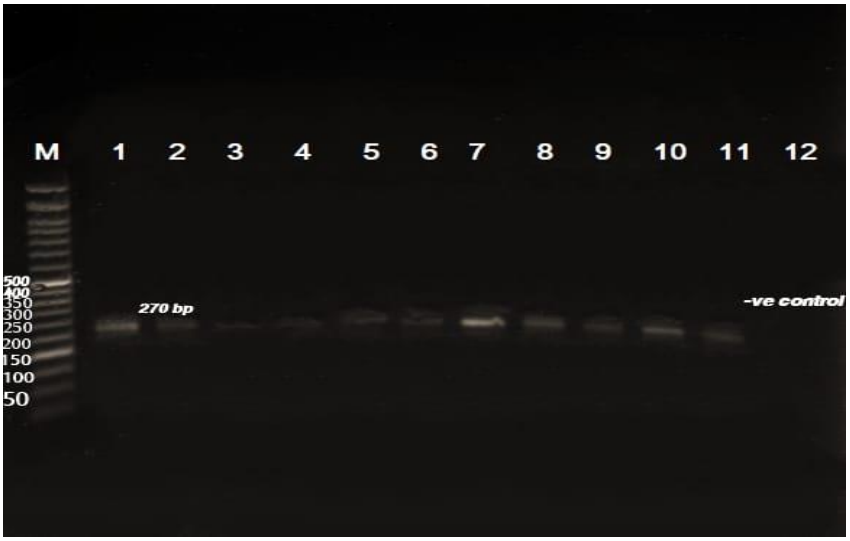

Photo.1: Results of PCR for testing gene encoding virulence factor of *T.pyogenes* (pyolysin) using primer (Pyolysin-F) for cows & buffaloes from dairy farms & farmers` houses

Lane M = Ladder marker

Lane 1 – 6 = Positive PCR products of DNA from *T.pyogenes* isolates from cows milk in this study with specific primer (Pyolysin-F)

Lane 7 - 11 = Positive PCR products of DNA from T.pyogenes isolates from buffaloes milk in this study with specific primer (Pyolysin-F)

Lane 12 = Negative control

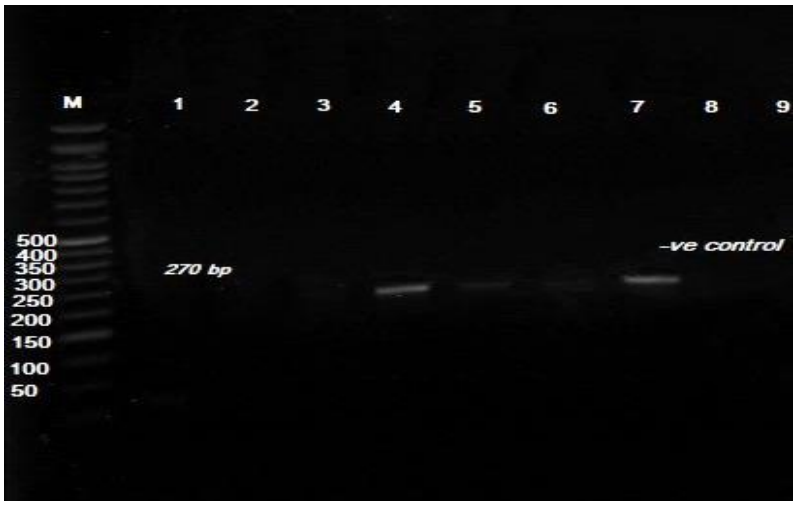

Photo 2: Results of PCR for testing gene encoding virulence factor of *T.pyogenes* (pyolysin) using primer (Pyolysin-F) for dairy shops` milk samples

Lane M = Ladder marker

Lane 1 = Negative PCR products of DNA from *T.pyogenes* isolates from buffalo`s milk in this study with specific primer (Pyolysin-F)

Lanes 2 and 3 = Negative PCR products of DNA from *T.pyogenes* isolates from sheep`s milk in this study with specific primer (Pyolysin-F)

Lanes 4 - 6 = Positive PCR products of DNA from *T.pyogenes* isolates from Dairy shops` milk in this study with specific primer (Pyolysin-F)

Lane 7 = Positive PCR products of DNA from *T.pyogenes* isolates from sheep` milk in this study with specific primer (Pyolysin-F)

Lane 8 = Negative control

DISCUSSION

Data presented in Table.1 showed the prevalence of SCM based on CMT and revealed that out of 30 of both cows and buffaloes` milk samples of dairy farms; 27 (90%) were positive. The lower incidences were estimated by Prasad *et al.* (2001) 61.32% ; Kader et al.(2002) 46.6% ; Maiti *et al. (*2003) 70.37% ; Sharma *et al.* (2004) 70.32% ; Sadek (2008) 59.05% ; Sayed and Abdel-Hafeez (2009) 31.82% ; Tripura *et al.* (2014) 51.8% ; Abebe *et al.* (2016) 67% ; El-Kholy *et al.* (2018) 44.83% and Mbindya *et al.* (2020) 76.6% . While, in buffaloes` milk, Lower incidence was described by Farah and Kaldes (1999) and Sadek (2008), as their results were 28.63% and 36.6%, respectively. The prevalence of SCM based on WST revealed an incidence rate of 50% at the cow level, nearly similar result of (54%) was obtained by Singh and Baxi (1988). Lower findings were estimated by Sayed and Abdel-hafeez (2009) and Islam *et al.* (2012) as they revealed animal-wise incidence of 33.64% and 25.5%, respectively. While, and Sadek (2008) obtained higher incidence of 60.95%. Regarding to buffaloes` milk samples out of 30, 14 (46.7%) samples were WST positive. Similar result 48.93% was detected by El-Balkemy *et al.* (1997). Lower finding 33.33% was obtained by Sadek (2008). However, Farah and Kaldes (1999) revealed a higher incidence of 63.3%. Concerning farmers` houses milk samples, the illustrated results showed that out of 30 cows` milk samples, 16 samples (53.33%) were positive. The current finding corroborate with those detected by Sori *et al.* (2005); Salih *et al.* (2011) and Mureithi and Njuguna (2016); as incidences of 52.78%, 52.8% and 54.2%. Lower incidences were obtained by Biffa *et al.* (2005) 33%; Tesfaye (2007) 7.3%; Molalegne *et al.* (2010) 28.2%; Sayed *et al.* (2011) 27.7%; Girma *et al.* (2012) 23.18%; Bangar *et al.* (2015) 46.35%; Belachew (2016) 5.3%; Sumon *et al.* (2017) 25% and Berhe *et al.* (2019) 27.89%. However, higher

incidences were obtained by Wahba *et al.* (2005); Sharma *et al.* (2008); Abramsen *et al.* (2014); Tolosa *et al.* (2015); Abebe *et al.* (2016); Devi and Dutta. (2018); and Mbindyo *et al.* (2020) that were 67%, 78%, 86.2%, 85%, 76%, 93.33% and 73.1%, respectively. Regarding WST% in farmers` houses, it is clear that out of 30 cows milk samples, 13 (43.3%) samples were WST% positive. Lower findings of 30.69%, 5.5% and 31.9% were obtained by Ghosh *et al.* (2004); Zahid (2004) and Sayed *et al.* (2011). However higher findings 69.2% and 67% were obtained by Awad and Abd-El-All (2003) and Wahba *et al.* (2005). Extremely a higher incidence was estimated by Bakr *et al.* (2019) 84%. In contrast, buffaloes farmers` houses results showed 22 (73.33%) out of 30 buffaloes` milk samples, were positive. Lower incidences were obtained by Sayed *et al.* (2011) and Yadav *et al.* (2019) that were 16.3% and 16.32%, respectively. Regarded to buffaloes` milk samples which were positive for WST, out of 30, 14 (46.7%) samples were positive. Similar result 48.93% was detected by El-Balkemy *et al.* (1997). Lower finding 33.33% was obtained by Sadek (2008). However, Farah and Kaldes (1999) revealed a higher incidence of 63.3%. Regarding sheep milk samples of farmers` houses, the recorded results based on CMT were 10 (33.33%) of 30 milk samples were positive. A lower incidence was obtained by Sadek (2008) 9.60%. However a higher incidence of 55.6% was shown by Moawad and Osman (2005). In addition, the positive sheep milk samples based on WST were 15 (50%) out of 30 samples. From the results illustrated in Table.2, data revealed the incidence of *T.pyogenes* in the dairy farm. Out of 30 cow`s milk samples, there were 18 (60%) positive for *T.pyogenes*. Lower incidences were estimated by Ribeiro *et al.* (2015) 45.1%; Alkasir *et al.* (2016) 28.6%; Momtaz *et al.* (2016) 36.50%; Tamai *et al.* (2018) 32.5% and Rezanejad *et al.* (2019) who detected that 32 (14.15%) out of 226 mastitic milk samples were positive for *T.pyogenes*. However, higher incidences were described by Hijazin *et al.* (2011) in Germany, they revealed that all 71 isolates (100%) have been identified as *T.pyogenes* and Mbindyo *et al.* (2020) 74.4%. With regard to buffaloes` milk samples of dairy farms in Table.2, the incidence of *T.pyogenes* was 18 (60%) out of 30 milk samples. Concerning the examined farmers` houses milk samples, it`s clear that 19 (63.33%) out of 30 in cow`s milk samples. A higher incidence was detected by Abdallah. (2016), who detected also that all 57 isolates (100%) were *T.pyogenes* and exhibited complete zone of hemolysis. Regarding buffalo`s millk samples of farmers` houses, results detected 18 (60%) positive samples out of 30 examined milk samples. In addition, 11 (36.67%) out of 30 sheep milk samples showed in Table.2 have the phenotypic properities of *T.pyogenes*. Data in Table.2 showed that 13 out of 30 dairy shops` raw milk samples were positive with incidence of 43%. The high incidence of *T.pyogenes* and mastitis in the present study may be due to inefficiency of milking personnel, and inadequate hygienic and sanitary measures applied during the milking process. Findings in Table.3 revealed that out of 30 milk samples in dairy farm cows` milk samples there were 27 positive samples for CMT, 15 positive milk samples for WST and 18 positive milk samples for *T.pyogenes*. In the contrary of buffaloes` milk samples of dairy farms, it was shown that out of 30 milk samples, the positive milk samples were 27 for CMT, 14 for WST and 18 for *T.pyogenes* . The obtained results in Table.3 illustrated that out of 30 cows` milk samples of farmers` houses, the positive samples were 17, 13 and 19 for CMT, WST and *T.pyogenes*, respectively. As regarding to buffaloes` milk samples, data showed that out of 30 milk samples, the positive milk samples were 22 for CMT, 14 for WST and 18 for *T.pyogenes*. In case of sheep`s milk samples, it is obvious that out of 30 milk samples, there were 10 positive CMT samples, 15 positive WST samples and 11 positive for *T.pyogenes* . In the present study, it was clear from photos 1 and 2 that, *plo* virulence gene was detected in 15 of 18 of examined strains in a percentage of 83.33 of the isolates. One of the most

important virulence genes is pyolysin (*plo*), an extracellular toxin, which is one of the first pathogenic factors detected in *T. pyogenes*. Although pyolosin was primarily identified as a hemolysin of red blood cells in a variety of animal species, its cytolytic effect has been demonstrated in several different host cells such as polymorphonuclear leukocytes (PMNS) and macrophages. Several studies have reported that all *T. pyogenes* strains encode pyolsin gene but the frequency of its expression is higher in pathogenic strains. From the results in photo.1, it is clear that 11 strains were isolated from dairy farms` and farmers` houses cow and buffalo milk samples which gave a positive results. The results illustrated in photo.2 revealed that 4 strains of dairy shops and sheep`s milk samples showed positive results for plo virulence gene. While, 1 buffalo and 2 sheep milk samples gave negative results. In comparison with the current study, a study was done by Bradely *et al.* (2015), all *T.pyogenes* isolates encoded plo with percentage of 100%. Another study performed by Abdallah (2016) revealed that all the 57 isolates were positive for plo encoding gene. In conclusion, raw milk samples of the present study revealed that most of the examined samples were contaminated with *T.pyogenes* and harbor plo gene which is responsible for the pathogenicity of the bacterium, so strict hygienic measures should be taken in consideration to improve the quality of raw milk and protect the economy of dairy industry.

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مدي تواجد ميكروب التروبيريلا بيوجينز في اللبن الخام

نجاح محمد سعد ، سحر محمود كمال ، أميرة جمال أبوصغير

Email: amira.gamal.abosgher@gmail.com Assiut University web-site: www.aun.edu.eg

تعتبر بكتيريا التروبيريلا بيوجينز من أخطر الميكروبات المنتشرة فهي تتسبب في حدوث التهاب الضرع الخفي الذي يعتبر من أخطر الأمراض المؤثرة اقتصاديا و صحيا لما تسببه من نقص في انتاج اللبن و تغيير في مكوناته الأساسية وقد تنتقل أيضا الي الانسان . ونظرا لأهمية هذه البكتيريا فقد تم تجميع عدد ١٨٠ عينة عشوائية من اللبن الخام من المزارع ومن بيوت المربين وكذلك مراكز بيع اللبن , وقد تم فحصها لعزل البكتيريا المعنية و احتمالية الاصابة بالتهاب الضرع الخفي. وبفحص عينات من مزارع الألبان و بيوت المربين وجد أن نسبة الاصابة بمرض التهاب الضرع الخفي باستخدام اختبارات CMTوWST, كانت ٩٠% لكل من عينات البان الأبقار والجاموس في المزارع وذلك باستخدام اختبار CMT. بينما كانت ٥٠% و ٤٦,٧% باستخدام اختبار WST . أما نسبة الاصابة في البان الابقار والجاموس والاغنام المجمعة من بيوت المربين الفلاحين كانت ايجابية بنسبة ٥٣,٣% ,٧٣,٣% و ٣٣,٣% على الترتيب . وعلى الصعيد الآخر كانت النتائج ايجابية في هذه العينات باستخدام اختبار الWST بنسبة ٣٣,٣% , ٤٦,٧ و ٥٠% على التوالي . أما في حالة نتائج الفحص البكتيريولوجي فكانت نسبة تواجد التروبيريلا بيوجينز عالية نسبيا, اذ كانت في مزارع الأبقار والجاموس ايجابية بنسبة ٦٠% لكل منهما . كما تبين أن نسبة العزل ضمن بيوت المربين هي : ٦٣,٣% , ٦٠% و ٣٦,٦٧% في الأبقار والجاموس والأغنام على التوالي . أما ما يخص عينات محلات الالبان تبين أن النتائج كانت ايجابية بنسبة ٤٣,٣% . وللتعرف على جين البيولايسن المسئول عن ضراوة هذه البكتيريا , فقد تم فحص ١٨ عترة معزولة من العينات المفحوصة , وذلك عن طريق تفاعل البلمرة المتسلسل تبين أن ١٥ عترة تحمل هذا الجين . مما يؤكد الأهمية الصحية والوبائية والاقتصادية لبكتيريا التروبيريلا بيوجينز, ومدى أهمية توافر الخدمات والرعاية الصحية والاهتمام بحيوانات المزارع وبيوت المربين وتطبيق الشروط الواجب اتباعها لمنع انتشار تلك البكتيريا الخطيرة مما يضمن سلامة الألبان.