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Milk quality characteristics of indigenous sheep breeds Boutsko, Frisarta and Karagouniko

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Ποιοτικά χαρακτηριστικά του γάλακτος αυτόχθονων ελληνικών φυλών προβάτων: Μπούτσκο, Φριζάρτα και Καραγκούνικο

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ABSTRACT. In this study, the chemical and microbiological characteristics of ovine milk from three indigenous Greek breeds, Boutsko, Frisarta and Karagouniko were examined, while reared in the semi mountainous areas. The milk yield of each sheep breed was recorded at the early, mid and late stages of lactation for two consecutive years. The average composition of the samples of ewe's milk used in this study was similar for protein, lactose and total solids among the three; however fat values were significantly lower ($p < 0.05$) in Frisarta milk compared to Boutsko and Karagouniko. Total viable cells of Boutsko and Karagouniko raw milk were lower ($p < 0.05$) than those of Frisarta. *Enterobacteria* detected in bulk raw milk from Boutsko and Frisarta breeds were in lower numbers ($p < 0.05$) compared to milk from Karagouniko breed. Somatic cells were also counted in lower numbers in milk collected from Boutsko and Karagouniko breeds compared to Frisarta. The percentage of detected as potentially pathogenic bacteria (coagulase negative staphylococci and enterobacteriae) was higher in the milk from Frisarta sheep milk.

Keywords: Boutsko breed, Frisarta breed, Karagouniko breed, microbiological quality, sheep milk, milk physicochemical characteristics.

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ΠΕΡΙΛΗΨΗ. Σκοπός της παρούσας μελέτης ήταν η διερεύνηση των φυσικο-χημικών χαρακτηριστικών και της μικροβιακής χλωρίδας του πρόβειου γάλακτος τριών αυτόχθονων Ελληνικών φυλών, του Μπούτσκου, του Καραγκούνικου και της Φριζάρτα, σε τρεις φάσεις της γαλακτικής περιόδου και κατά δύο συνεχείς γαλακτικές περιόδους. Επίσης, εκτιμήθηκαν η ποσότητα του γάλακτος κάθε φυλής κατά τη γαλακτική περίοδο, οι εποχιακές διακυμάνσεις του ποσοστού της πρωτεΐνης και του λίπους στο γάλα των τριών φυλών. Ταυτόχρονα, μετρήθηκε η ολική μεσόφιλη χλωρίδα, ο ολικός αριθμός των ψυχρότροφων βακτηρίων, τα εντεροβακτηριοειδή και έγινε επιμέτρηση των σωματικών κυττάρων σε ατομικά δείγματα προβατίνων των τριών υπό μελέτη φυλών. Η μέση τιμή της πρωτεΐνης, της λακτόζης και των ολικών στερεών του γάλακτος ήταν χωρίς στατιστικώς σημαντικές διαφορές, ενώ το ποσοστό του λίπους ήταν αυξημένο στο γάλα των φυλών Μπούτσκο και Καραγκούνικο σε σχέση με το γάλα της φυλής Φριζάρτα ($p < 0.05$). Η ολική μεσόφιλη χλωρίδα στο γάλα των φυλών Μπούτσκου και Καραγκούνικου ήταν σημαντικά μικρότερη της αντίστοιχης του γάλακτος της φυλής Φριζάρτα ($p < 0.05$), τα εντεροβακτηριοειδή ανιχνεύθηκαν σε μικρότερους αριθμούς στο γάλα των φυλών Μπούτσκο και Φριζάρτα συγκρινόμενα με του Καραγκούνικου, ενώ ο αριθμός των σωματικών κυττάρων μετρήθηκε χαμηλότερος στο γάλα των φυλών Μπούτσκο και Καραγκούνικο σε σχέση με αυτόν της φυλής Φριζάρτα. Το ποσοστό των απομονωθέντων δυνητικά παθογόνων βακτηρίων (κοαγκουλάση αρνητικοί σταφυλόκοκκοι και εντεροβακτηριοειδή) ήταν στατιστικώς σημαντικά αυξημένο ($p < 0.05$) στο γάλα της φυλής Φριζάρτα.

Λέξεις ευρετηρίασης: μικροβιολογικά χαρακτηριστικά, πρόβειο γάλα, φυλή Καραγκούνικο, φυλή Μπούτσκο, φυλή Φριζάρτα, φυσικο-χημικά χαρακτηριστικά γάλακτος.

INTRODUCTION

Sheep production is of particular economic interest in certain areas of the world, as it is a sustainable resource with excellent possibilities of economic profitability and demographic stability, of special importance for arid, semi-arid and other unbendable regions. Especially, in the mountainous or semi-mountainous areas of the Mediterranean basin, sheep are reared under an extensive or semi-extensive management regime prioritizing autochthonous breeds that are valuable in preserving genetic variability, while production costs are held down by the appropriate use of natural resources. The foods produced, namely milk and meat (from the young animals) are from a nutritional point of view of excellent quality (Boza, 1993; Sanz Sampelayo et al., 2007).

Sheep breeders may be prompted to maintain all quality aspects of their products and to adopt an advanced farming system. In the Mediterranean region, the percent of pasture land is decreasing as well as the percent of the environment dedicated to the farming system. This evolution may result in a decrease of the “typicity” and quality of some

cheeses (Morand-Fehr et al., 2007). The chemical composition of milk, in terms of fat content, depends on dietary (composition and availability), animal (breed, lactation stage, body condition) and environmental (especially cold and heat stress) factors (Nudda et al., 2014). Dietary factors that affect milk fat and protein content and cheese yield have been reviewed previously in detail (Pulina et al., 2006; Giannenas et al., 2011, Nudda et al., 2014).

Data on quality characteristics of milk of certain indigenous Greek breeds are rather scarce. Kondyli et al. (2012) investigated the basic composition, fat soluble vitamins, fatty acids (including CLA) and the microbiological quality of the Boutsko and Karamaniko native sheep Greek breeds (Kondyli et al., 2012). As sheep milk is mainly dedicated to cheese-making, the scope of this paper was to compare milk composition and milk bacterial flora from three different local breeds such as the Boutsko, Karagouniko and Frisarta that are raised in the areas of Western and Central Greece, with special focus on the analysis of seasonal variations of fat and protein content and bacterial flora of their milk.

MATERIALS AND METHODS

1. Rearing system and Milk samples

Feeding regimes were based on the rearing system of each breed with Boutsko ewes being fed mainly by grazing in mountainous pastures (area of Tzoumerka), Karagouniko ewes given long hours of daily forage allowance for grazing in semi-mountainous pastures of Western Greece, supplemented with lucerne hay, wheat straw and concentrate feed (only for the first 3 months of lactation), and Frisarta ewes given long hours of daily forage allowance for grazing in semi-mountainous pastures of western Greece, supplemented with lucerne hay, wheat straw and concentrate feed (for the first 5-6 months of lactation). The suckling period was 60 days in both Boutsko and Karagouniko lambs, and 40 days for the Frisarta lambs on average, whereas ewes were hand - milked once every day during this period, while after the suckling period the ewes were hand - milked twice daily. Boutsko is regarded as the most adopted breed reared in the mountainous areas of Greece, Karagouniko as the most outnumbered adopted breed in the semi-mountainous areas and Frisarta is a new breed reared in the area of Western Greece with improved high milk yield phenotype.

All animals had been drenched with an antiparasitic drug and vaccinated against clostridial infections 3 weeks before lambing; no ewe received antimicrobial agents during lactation. All procedures were performed by the same team members on all herds throughout the project.

A total of 144 raw bulk milk samples were obtained from 4 flocks belonging to each of the three different breeds of sheep, in the areas of Western and Central Greece, for two consecutive years. These samples were collected aseptically from the farm tank every second month (three times annually, from January/February, April/May, June/July) and were used to analyze the chemical composition of milk and the determination of Total Viable Count (TVC), *Enterobacteriaceae* and Total Number of Psychrotrophic Bacteria (TNPB). Additionally, secretion samples of 10-15 ml individually from both mammary glands of each ewe were collected, representing 5% of the animal population at random, of the aforementioned flocks, following the proper procedures (Fthenakis, 1994), into a sterile container, during the mid-period of lactation and were examined

for pathogenic bacteria. In the same individual samples the number of nucleated cells was also determined. Samples were delivered to the laboratory of Animal Health and Food Hygiene and Quality at TEI of Epirus (Arta) in a cool box at 4°C immediately after collection and tested upon arrival.

2. Milk Production and milk chemical composition

The milk yield of each sheep breed was recorded at the three stages of lactation period (early, January/February, mid, April/May, late, June/July) for two consecutive years. Milk samples were analysed for fat, protein, lactose, total solids, and non-fat solids by NIRS using MilkoScan 4000 (FOSS Electric, Integrated Milk Testing™, Denmark). Milk acidity was measured by a portable pH meter BT-600 (BOECO, Germany).

3. Microbiological analysis

Samples from the farm bulk tanks were analyzed for their microbiological quality as well as the prevalence of bacterial pathogens in individual samples, for estimating udder's hygienic status in the herd. For this purpose TVC and *Enterobacteriaceae* counts were determined by using the TEMPO method (Crowley et al., 2009; Owen et al., 2010). The TEMPO® system (bioMérieux, France) is a semi-automated analyser based on Most Probable Number (MPN), where the microbial growth is indicated by an increase/or decrease in fluorescence. Total aerobic bacteria and *Enterobacteriaceae* counts were determined by TEMPO® TVC and TEMPO® EB kits (bioMérieux, France) respectively, according to the manufacturer's instructions. The TEMPO-TVC kit was used for the enumeration of total aerobic bacteria, and the card was incubated at 35°C for 40–48 h (Crowley et al., 2009). *Enterobacteriaceae* was enumerated using the TEMPO-EB kit, the card was incubated at 35°C for 22–27 h (Owen et al., 2010). Serial decimal dilutions of the milk samples in Ringer's solution up to 10⁻⁴ were performed and cultures were prepared on plate count agar (Merck) for enumeration of TNBP as described by standard methods of the American Public Health Association. The incubation conditions were at a constant temperature of 6.5 ± 0.5°C for 10 days (Downes, 2001).

For the isolation of pathogenic bacteria, 10

Table 1. Mean chemical composition of ovine milk of three Greek breeds

	Greek breeds		
	Boutsko (n = 48)	Karagouniko (n = 48)	Frisarta (n = 48)
Milk, (g/d)	711.1 ^c	966.5 ^b	1280.0 ^a
pH	6.82 ± 0.15	6.76 ± 0.10	6.89 ± 0.19
Fat (g/kg)	85.76 ± 13.50 ^a	83.53 ± 14.71 ^a	61.23 ± 6.03 ^b
Protein (g/kg)	59.52 ± 7.68	56.23 ± 4.11	54.34 ± 5.20
Lactose (g/kg)	45.99 ± 4.87	45.00 ± 2.85	46.47 ± 3.56

a,b,c: values in the same row with different superscript) differ significantly at $p < 0.05$

μl from the secretion samples were plated onto Columbia 5% sheep blood agar and were incubated aerobically at 37°C for up to 72 h. The isolated bacteria were identified by using conventional microbiological techniques (Barrow and Feltham, 1993). In the individual samples the number of nucleated cells was determined by the application of California Mastitis Test (CMT), as described by Fthenakis (1995) for ewe's milk and also for the somatic cell counts, determined using DeLaval somatic cell counter (DeLaval International AB, Tumba, Sweden).

4. Statistical analysis

Data on milk production and composition were analyzed as repeated measurements by ANOVA in the General Linear Model of the SPSS v.20.00, statistical package (SPSS Inc., Chicago, USA). Data on fatty acids, bacterial counts and SCC were analyzed by one way ANOVA; as for above, each breed was considered to be the statistical unit. The homogeneity of the variances was tested by Levene's test. The Tukey's multiple comparison test was carried out to assess any significant differences at a probability level of $P < 0.05$ between the experimental groups, when a significant effect of treatment was detected by the ANOVA.

RESULTS AND DISCUSSION

The increasing attention of consumers to the nutritional and health aspects of food has recently shifted the focus of dairy sheep producers towards

Table 2. Lactation stage variation of milk of Boutsko ewes

	Early (n = 16)	Mid (n = 16)	Late (n = 16)
pH	6.78 ± 0.18	6.81 ± 0.13	6.90 ± 0.12
Fat (g/kg)	80.13 ± 22.08 ^b	83.96 ± 5.02 ^{a,b}	95.75 ± 7.06 ^a
Protein (g/kg)	55.96 ± 7.93 ^b	59.15 ± 4.80 ^{a,b}	64.19 ± 8.49 ^a
Lactose (g/kg)	43.30 ± 3.07 ^b	46.08 ± 4.31 ^a	42.03 ± 4.44 ^b

a,b: values in the same row with different superscript differ significantly at $p < 0.05$

the achievement of an appropriate milk lipid composition. Furthermore, consumers are demanding dairy products with a special flavor associated with the territory where the animals live. Table 1 reports the mean values of chemical composition of raw bulk milk samples of the three different breeds collected consecutively for 2 years. In general, these contents are not constant and vary depending on breed, stage of lactation, age and season (Yabrir et al., 2013). The average composition of the samples of ewe's milk of three breeds used in this study was similar for protein, lactose and total solids among the three; however fat values were significantly lower in the Frisarta milk compared to both the Boutsko and Karagouniko breeds (Table 1). Our findings are in agreement with Haenlein (2002) who reported a negative correlation between milk yield and composition. In our work the Frisarta breed gave higher milk yield values compared to the other 2 breeds, presented significantly lower milk fat values. Our results for Boutsko breed are also in agreement with previous reports concerning the levels of the basic constituents in milk of the Boutsko breed (Voutsinas et al., 1988; Kondyli and Katsiari, 2002; Kondyli et al., 2012). Similar results about chemical composition characteristics of the Boutsko breed have been also reported for the Karamaniko breed (Kondyli et al., 2012), however very limited data are available for milk of the Frisarta or Karagouniko breeds in the literature.

As far as the physico-chemical characteristics are concerned, pH milk values were not affected by sheep breed (Tables 1-4). Though, similar values have been previously reported for the Boutsko breed

Table 3. Lactation stage variation of milk of Karagouniko ewes

	Early (n = 16)	Mid (n = 16)	Late (n = 16)
<i>pH</i>	6.76 ± 0.05	6.70 ± 0.09	6.82 ± 0.11
<i>Fat (g/kg)</i>	74.30 ± 11.19 ^b	80.48 ± 13.43 ^{a,b}	92.24 ± 16.88 ^a
<i>Protein (g/kg)</i>	53.17 ± 3.51	55.05 ± 3.33	59.30 ± 3.32
<i>Lactose (g/kg)</i>	47.34 ± 1.86 ^a	45.40 ± 1.92 ^{a,b}	43.21 ± 1.68 ^b

a,b: values in the same row with different superscript differ significantly at $p < 0.05$

Table 4. Lactation stage variation of milk of Frisarta ewes

	Early (n = 16)	Mid (n = 16)	Late (n = 16)
<i>pH</i>	6.85 ± 0.24	6.89 ± 0.15	6.98 ± 0.09
<i>Fat (g/kg)</i>	56.00 ± 5.48 ^b	61.50 ± 7.98 ^a	65.72 ± 6.62 ^a
<i>Protein (g/kg)</i>	50.91 ± 4.52	56.18 ± 4.22	57.80 ± 4.63
<i>Lactose (g/kg)</i>	48.68 ± 2.19 ^a	45.49 ± 3.02 ^{a,b}	43.72 ± 1.63 ^b

a,b: values in the same row with different superscript) differ significantly at $p < 0.05$

(Kondyli et al., 2012) or milk of other sheep breeds in Mediterranean or Balkan countries (Pavić et al., 2002; Park et al., 2007; Kuchtlík et al., 2008; Hilali et al., 2011; Yabrir et al., 2013).

The rearing season significantly affected the fat and lactose content in ewe milk of the Karagouniko and Frisarta breeds (Tables 3&4). Milk composition characteristics of the Boutsko breed regardless of the strong tendency of lactose decrease and fat increase from early to late lactation period remained in high fat and protein levels all season (Table 2). Similarly, changes in the physico-chemical characteristics of ewe's milk during the stage of lactation have been reported by several authors (Pavić et al., 2002; Bianchi et al., 2004; Kuchtlík et al., 2008; Yabrir et al., 2013). The fat percentage increased significantly as lactation progressed.

In our study, the enumeration of mesophilic bacteria (TVC) fluctuated from $10^1 - 10^6$ cfu/ml and the psychrotropic bacteria (TNBP) from $10^1 - 10^6$ in the bulk milk (Table 5). Same studies with the differentiation that the samples immediately originated from ewes' mammary glands showed $10^2 - 10^6$ cfu/ml and $10^2 - 10^4$ cfu/ml respectively (Fotou et al., 2011). Bramley and McKinnon (1990) reported that the number of psychrotrophs should represent about 10-50% of total bacteria count, which is the case with our results. The measurement of *Enterobacteriaceae* ranged from $10^1 - 10^6$ cfu/ml. The values of these kinds of bacteria are significantly lower in milk samples for the Boutsko breed (Table 5) followed by the Karagouniko and Frisarta breeds. These results were in accordance with the prevalence of some bacteria such as CNS, *Enterobacteriaceae*,

E. coli and *Streptococcus spp.* (Table 6) in individual raw ewe's samples as well as with CMT and SSC (Table 7). In general, the microbial quality of ewe milk based on TVC values, was at acceptable levels according the EU Regulations (EU, 2004): for milk originating from small ruminants, which will undergo pasteurization must not exceed a Total Viable Count (TVC) in 30 °C count of 1.5×10^6 , while milk from the same origin that will not undergo any kind of thermal process should not exceed a TVC count of 5×10^5 . Possible reasons for the high load of *Enterobacteriaceae* could be improper conditions in farm hygienic status or inadequate milk storage conditions at farm level. On the other hand, the lower values of TVC and EB in milk samples of the Boutsko in comparison to the other two breeds might be attributed to its excellent adaptation to the Greek mountainous environmental conditions. Similar studies, with bulk milk samples, for the Boutsko breed have shown TVC $< 10^6$ cfu/ml (Kondyli et al., 2012).

The prominent pathogenic group in our findings was CNS and its prevalence was between 31.8% and 62.1% with the main tolerance to the Boutsko breed (Table 6) Several studies pointed to CNS as the main etiological agent of small ruminant intramammary infection (Leitner et al., 2001; Bergonier and Berthelot, 2003; Contreras et al., 2007; Nunes et al., 2008; Guaraná et al., 2011). Moreover correlations studies between CMT, SCC and the isolation of bacteria from the mammary secretions have shown that isolation of bacteria was associated with increased SCC and CMT in sheep. Bacterial infection in ewe's mammary gland has been shown to increase SCC (Dulin et al., 1983; Green,

Table 5. Enumeration of different microbiological parameters detected in bulk raw ewe's milk samples

(cfu / ml)	Boutsko			Karagouniko			Frizarta		
	Min	Max	Average ± SD	Min	Max	Average ± SD	Min	Max	Average ± SD
TVC ¹	8.0 x 10 ¹	2.7 x 10 ⁶	1.2 x 10 ⁶ ± 5.6 x 10 ⁵	8.5 x 10 ³	1.6 x 10 ⁶	1.4 x 10 ⁶ ± 8.7 x 10 ⁵	9.0 x 10 ⁵	4.9 x 10 ⁶	1.7 x 10 ⁶ ± 6.1 x 10 ⁵
EB ²	6.0 x 10 ¹	1.4 x 10 ⁵	6.6 x 10 ⁴ ± 4.6 x 10 ^{4b}	1.2 x 10 ²	2.3 x 10 ⁶	5.9 x 10 ⁵ ± 3.1 x 10 ^{5a}	4.0 x 10 ²	5.5 x 10 ⁵	8.4 x 10 ⁴ ± 1.9 x 10 ^{4b}
PS ³	1.2 x 10 ¹	8.0 x 10 ⁵	5.8 x 10 ⁵ ± 1.9 x 10 ⁵	2.8 x 10 ²	2.5 x 10 ⁶	6.8 x 10 ⁵ ± 1.4 x 10 ⁵	3.2 x 10 ²	1.6 x 10 ⁶	8.8 x 10 ⁵ ± 3.4 x 10 ⁵

1: Total Viable Count, 2: Enterobacteriaceae, 3: Psychrotrophic. a, b: values in the same row with different superscript) differ significantly the level of p<0.05

Table 6. Percentage of detected pathogenic bacteria in individual raw ewe's milk samples

of positive samples	Boutsko (n = 16)			Karagouniko (n = 4)			Frizarta (n = 13)		
	Bacterial strains	No of samples	Positive samples %	Bacterial strains	No of samples	Positive samples %	Bacterial strains	No of samples	Positive samples %
<i>Staph. aureus</i>	22	ND ^d	0	10	ND	0	29	ND	0
CNS ^e	22	7	31.8	10	4	40	29	18	62.1
Enterobacteriaceae	22	4	18.1	10	1	10	29	8	27.6
<i>E. coli</i>	22	1	4.5	10	1	10	29	ND	0
<i>Streptococcus spp</i>	22	ND	0	10	ND	0	29	2	6.9

d: Not detected, e: Coagulase Negative Staphylococci

1984). In the current study all our individual milk ewe's samples were 0-1 for CMT and 0.2-0.34 x10⁶ for SCC. In sheep, the SCC from uninfected glands ranges from 0.26 to 1.58 x10⁶ SCC/ml (Fthenakis, 1994; Burriel, 1997; Kiossis et al., 2012; Petridis et al., 2012). Cut-off points ranging between 0.3 and 1.7 x 10⁶ SCC/ml in sheep (Green, 1984; Fthenakis, 1995) have been used to differentiate between infected and non – infected glands. Also, the CMT score is positively correlated with SCC (Kalogridou-Vassiliadou et al., 1992; Contreras et al., 1996).

In ewes, the majority of proposed threshold values for discriminating between healthy and infected halves are lower than 500 x 10³ cells/ml (González-Rodríguez et al., 1995; Pengov, 2001; Nunes et al., 2008). However, higher values, up to 1.500 x 10³ cells/ml, have been suggested by some authors (Fthenakis et al., 1991; Mavrogenis et al., 1995; Lafi, 2006). Several studies evaluated CMT as an indirect test for mastitis. In ewes, most of these studies suggested a cut-off of 1 unit for the CMT to identify infected glands (Hueston et al., 1986; Clements et al., 2003; Nunes et al., 2008; Peixoto et al., 2010). Strong correlations between the CMT and bacterial exams (73.2%) (Peixoto et al., 2010) have been reported using the CMT score of 1 as a threshold. Strong correlations between CMT and SCC (r = 0.65; (Suarez et al., 2002)), (r = 0.75), (r = 0.82; (Della Libera et al., 2011)), (r = 0.84; (Gomes et al., 2006)) have been described as well in sheep.

CONCLUDING REMARKS

This paper deals with several quality characteristics of milk of three different indigenous sheep breeds reared in Greece. Differences in the composition and quality of sheep milk of three different sheep breeds are noteworthy. Milk yield was higher in Frisarta, intermediate in Karagouniko and lower in Boutsko breed. Boutsko breed (extensively grazing) showed no significant differences for fat, protein, lactose and solids-non-fat contents during early, mid or late lactation period. However, the milk from the Karagouniko ewes (mostly grazing), and Frisarta (mostly indoor system) appeared to be more influenced by seasonal variation, than milk from Boutsko ewes. The microbiological quality of ewe milk of both Boutsko and Karagouniko breeds was better than that of Frisarta sheep milk, taking into

Table 7. Enumeration of somatic cells in individual raw ewe's milk samples

	<i>Boutsko</i> (n = 14)	<i>Karagouniko</i> (n = 12)	<i>Frizarta</i> (n = 12)
CMT	0 – 1	0 – 1	0 – 1
SCC (cells x 10 ³ / ml)	210.6 ± 67.9 ^b	239.0 ± 72.1 ^{a,b}	349.0 ± 68.2 ^a

a,b: values in the same row with different superscript) differ significantly at the level of p<0.05.

account the total viable counts and enterobacteriaceae, as well as SCC and CMT. This study showed that all parameters were affected by breed (except pH) and season (except for the Boutsko breed). The lower values of TVC and EB, as well as SCC and CMT test in milk samples of the Boutsko breed in comparison

to the other two breeds may be attributed to its better adaptation to the Greek environmental conditions.

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CONFLICT OF INTEREST STATEMENT

None of the authors have any conflicts of interest to declare regarding this work. ■

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