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Relationship of polymorphism of untranslated region (5-UTR) of the insulin-like growth factor (IGF-1) gene with milk production and growth traits in local black goats

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Abstract---The study was conducted on a sample of 34 local goats in a private field of a breeder in Canaan sub-district / Diyala governorate. Laboratory work was conducted in the laboratory of the College of Agriculture - University of Diyala and the Office of Scientific Advancement - Al-Harithiya - Baghdad- Iraq for the period from 13/11/2021 to 1/5/ 2022 with aim of studying the genetic polymorphism of the untranslated region (5-UTR) of the IGF-1 gene and its relationship to milk traits (daily milk production - DMY and total milk production - TMY) and growth traits (birth weight -BWT, weaning weight -WWT and weight gain pre-weaning-WG). The results of the study showed a highly significant ($P < 0.01$) between the genotypes, as two genotypes of 5-UTR were observed, namely, GG and AG, with frequency distribution ratios of 91% and 9%, respectively, while the frequency of the G allele was more (94%) compared frequency A allele (6%) in the studied sample. It was noted that there were highly significant differences ($P < 0.01$) between the genotypes of 5-UTR in the sample of the studied animals for daily and total milk production, as the genotype GG recorded the highest production for the daily and total milk production trait (0.343 and 268.62) kg, respectively compared with the genotype AG (0.213, (167.02 kg) respectively. The results of the study also showed that there were non- significant differences between the genotypes in BWT and WG, while there were highly significant differences ($P < 0.01$) for WWT, as the genotype outperformed GG (14.39) kg on genotype AG (10.20) kg.

Keywords---IGF-1 gene, milk production, growth traits, local goats.

Introduction

Goats are distinguished from farm animals in that their management and nutrition are simple, as well as their birth to twins, early sexual puberty, metabolic efficiency and long productive life, as well as their efficiency in using pastures and eating feed that other animals may not eat and its cheap price compared to sheep (Al-Qass et al., 1993). In Iraq, there are three breeds of goats: local black goats, mountain black goats and the miraaz goats (Al-Jalili et al., (1984). One of the main objectives of raising this animal is to produce meat by increasing the proportion of twins in births in the first place. The gene for insulin-like growth factor (IGF-1) is a mediator of growth hormone (GH), which is one of the effective genetic markers for growth traits in farmed animals (Estany et al., 2007). As many researchers turned to study the genetic polymorphism of genes and know their association with growth traits (Jade et al., 2011). IGF-1, in relation to the growth hormone gene, affects growth traits (Ge et al., 2001) IGF-1 is also a mediator of many biological effects, for example it stimulates muscle formation, increases glucose uptake, increases fat synthesis, and stimulates progesterone production. In granulosa cells and interferes with cell proliferation (Yurnalis et al., 2017).

The IGF-1 is secreted during the estrous cycle from the uterus in goats and its concentration in the blood plasma increases during estrus (Wathes et al., 1998), and that this secretion is positively affected by the secretion of insulin-like growth factor (IGF). Estrogen hormone, as it activates the secretion of IGF-1 from the uterus (Todd et al.,2010), and the latter is necessary for the secretion of (LH), which is at its highest level in estrus, as the number of estrogen receptors increases in the cells of the uterus, and through its association with it increases the mRNA formation of IGF-1 in utero (Wathes et al., 1998) and then increase of IGF-1 gene expression in utero independent of growth hormone (Jones and Clemmons, 1995). IGF-1 levels peak during the estrus phase coincides with the peak of the hormone LH that precedes ovulation and is higher than it during the luteal phase (Hashizume et al., 2000). It was found that the amount of feed intake affects the secretion of IGF-1 and its formation in the liver, and that the animal was exposed to poor energy nutrition for a period of one It was short in the beginning of the reproductive season and significantly affects the ovarian activity.

Materials and Methods

The study was conducted on a sample of 34 local black goats in Diyala Governorate / Kanaan in the field of a goat breeder. Laboratory work was conducted in the laboratory of the College of Agriculture - University of Diyala - Iraq for the period from 11/13/2021 to 5/1/2022/ in order to determine the genotypes of the untranslated region (5-UTR) of the IGF-1 gene and its relationship to daily and total milk production (DMY and TMY) and growth traits (birth weight -BWT, weaning weight- WWT and weight gain pre- weaning WG). TMY of the studied goats for one production season was calculated based on the DMY, which was measured weekly for each a goat by manual milking method by

isolating the new-born from their dams at night and for a period of 12 hours from eight in the middle of the night until seven in the morning, in order for the goats to be milked in the morning depending on the morning circuit, and then TMY was estimated (ICAR, 2004), based on the following equation (Gootwine and Pollott, 2000):

$$\text{TMY} = (\text{T}_1 - \text{T}_0) \text{M}_1 + \sum^{11} (\text{T}_r - \text{T}_{r-1}) (\text{M}_r + \text{M}_{r-1}) / 2$$

TMY = total milk production.

- T0 = date of birth.
- T1 = the date of the first measurement.
- M1 = the first measurement (amount of milk in kg).
- Tr = the date of measurement for that week.
- Tr-1 = measurement date for the previous week.
- Mr = the measurement in that week (amount of milk in kg).

The weight of the dams at birth was taken on a scale of 150 kg. The WWT was measured after three months with a scale of (150 kg), and then WG was calculated through the difference between WWT and BWT according to the following equation: $\text{WG} = \text{WWT} - \text{BWT}$. Blood samples were drawn from the jugular vein, a 5 ml medical syringe was used after cleaning the jugular vein area and sterilizing the area with 70% ethyl alcohol. Until the DNA extraction process, DNA was also extracted from the blood according to the instructions of the diagnostic kit (kit) supplied by the Taiwanese company FAVORGEN. Primers of the IGF-gene were identified, which were used to multiply 5-UTR of IGF-1 gene with a size of 294 base pairs (Naicy et al., 2017) (Table 1).

Table 1
Primer sequences of position 5-UTR to IGF-1 gene

Gene name	Size (bp)	Primer sequence	position	Annealing
IGF-1	294	F:5-TGAGGGGAGCCAATTACAAAGC-3 R: 5- CCGGGCATGAAGACACACACAT- 3	5-UTR	55

(Naicy et al., 2017)

The required segment amplification program was also determined using PCR technique according to Table (2). Table (3) shows the reaction materials that were used to amplify the required segment of the IGF-1 gene, which has a size of 294 base pairs. The genotypes of the studied region were determined using the SNP.

Table 2
Program of PCR technique

stages	Temperature C	Time	No. of Cycles
Initialization Denaturation	95	3 Minutes	1
Denaturation	94	30 Seconds	35
Annealing	55	30 Seconds	

Extension	72	30 Seconds	
Final Extension	72	5 Minutes	

Table 3
PCR reaction mixture

Chemical material	Master Mix (μl)	Primer (μl)	DNA Templet (μl)	Distilled water (μl)	Final volume (μl)
Volume	12.5	R-1 F-1	5	5.5	25

The data on milk production and growth traits were analyzed using the General Linear Model (GLM) method within the SAS (2012) statistical program according to the following mathematical model :

$$Y_{ijklm} = \mu + G_i + T_j + S_k + M_l + e_{ijklm}$$

Since:

Y_{ijklm} : watch value m .

μ : over all mean.

G_i : Effect of Genotypes (GG and AG).

T_j : effect of birth type j (single, twin).

S_k : effect of sex of newborn k (male, female).

M_l : effect of the month of birth (November, December, January, February).

e_{ijkl} : The random error, which is normally distributed with an average of zero and a variation of σ^2_e .

The Chi-square-x2 test was used to compare the percentages of the genotypes distribution of the studied gene mutation in 5-UTR and the significant differences between the means were compared using Duncan's test (1955). The law is below:
Frequency of the first allele:

$$2 \times \text{No. of Homozygous} + 1 \times \text{No. of Heterozygous}$$

$$P_A = \frac{\text{Frequency of the first allele}}{2 \times \text{Total number of sample}}$$

As for the frequency of the second allele, it is according to the equation:

$$q_B = 1 - P_A$$

Results and Discussion

The studied fragments of 5-UTR of IGF-1 gene were amplified using PCR technical. The gene fragment (294 bp) as shown in Figure (1) with agarose gel (1%) and TBE 1X buffer.

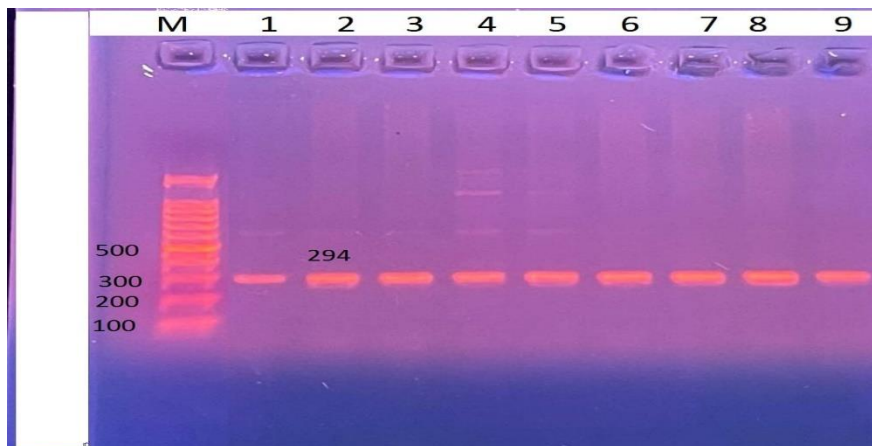


Figure 1. The product of the electrophoresis process of the 5-UTR region of the IGF-1 gene with agarose gel (1%) and TBE 1X buffer, as the size of the amplified piece was 294 bp

Polymorphism of the 5-UTR of IGF-1 gene

A mutation was identified within the site (A182G), as the base changed from A to G, which represents the homologous replacement mutation (Transition) (Fig. 2). The wild allele A and the mutant allele G were in the presence of two genotypes, GG and AG, and did not appear in the studied sample in 5-UTR of wild AA genotype. The results of this study were in agreement with what was stated by (Thomas et al., 2017) who observed the presence of two genotypes (GG) and AG) in 5-UTR in two breeds of Indian domestic goats (Attappady Black and AG). Malabari). In another study, two genotypes (GG and AG) were found in the region same (5-UTR) in two breeds of Chinese goats (Guanzhong and Xinony saanen) (Deng et al., 2010). In Sarda sheep, it was found that there are three Genotypes: GG, GA, and AA at position 857 (Di Stefano., 2017 and Sebastiano et al., 2020). Figure (3) shows the target segment sequence for the 5-UTR, where we find a change wild allele (A) to mutant allele (G) at sequence 182 (shaded in blue) from the beginning of the forward initiator (in yellow) and represents a homozygous substitution mutation of the purine base substitution A to the base of the purine G.

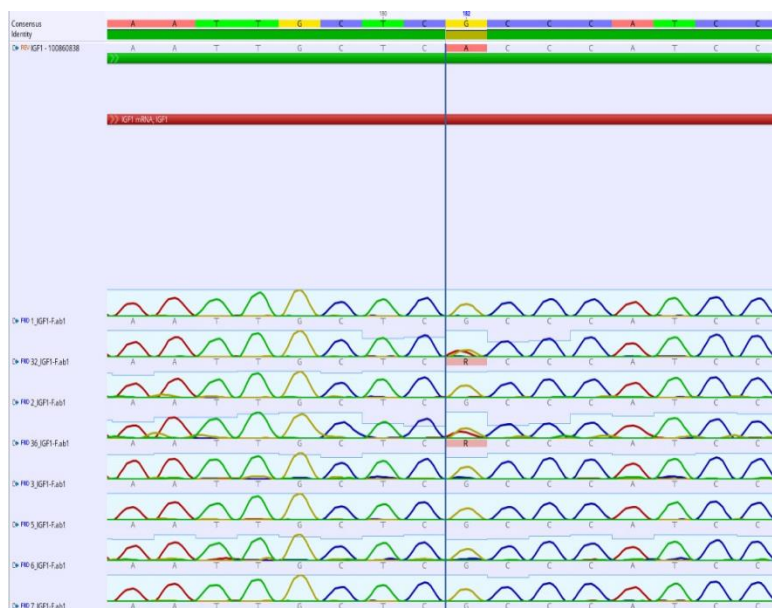


Figure 2. Genetic polymorphism of the 5-UTR within Site 182.

TGAGGGGAGCCAATTACAAAGCTGCCTGCCCTTTCAGGTTCTAGGAAATGAG
ATCATTCCCCTCACTTGGCAACCAGGACGAGGGGTCATCCCAGCGCCGTCTTCC
AGTCTAGTTTACCCAGTCGTTTGAGGGTTAAAATCATAGAGTATGCTTGAGATG
TCTTTTTTTCATTCTTTTTTTAAATTTTGTCTTGCTCTGGAATATAAAATG
CTCACCACATCCTCCACGAATATTCCTTTCATACGGGTAAGGTGTATTAGCAGATG
TGTGTGTCTTTCATGCCCGG

Figure 3. Mutation site (A182G) of the PCR product sequence segment (294 base pairs) for the 5-UTR region of the IGF1 gene in goats (*Capra hircus*) (GenBank accession number: HQ731040.1).

Distribution of genotypes and alleles frequencies of the studied region of IGF-1 gene

The distribution ratios for the GG and AG genotypes were 91.0 and 0.09, respectively, and there were highly significant ($P < 0.01$) differences in the distribution ratios, while the allelic distribution for each of the G and A allele reached 0.94 and 0.06, respectively, from 5'-UTR of IGF-1 gene (Table 4), as the frequency of the G allele was high compared to the A allele in the studied sample. The increase of the G allele may be due to the number of animals in this study or to a non-direction selection of the G allele, which showed a clear relationship with the studied productive traits, which will be mentioned later. We find from the study ratios in agreement with a number of studies, as most of them indicated a higher percentage of GG genotypes compared to AG genotypes, as (Thomas et al., 2017) indicated during his study on two Indian goat breeds that the frequency of GG genotypes amounted to 0.62% compared to AG genotype 0.38%. And (Deng et al., 2010) in a study on two breeds of Chinese goats (Guanzhong and Xinony saanen), with a higher percentage of GG genotype (0.64%) than AG genotype (0.36%). This is what was observed by (Sebastiano et al., 2020) in a study on Sarda sheep, where the percentage of genotypes CC 0.85 was the highest for the

rest of the structures CG and GG (0.11, 0.4), respectively. And (Di Stefano., 2017) in a study on the Sarda sheep breed, the genotypes GG, AG and AA were (0.89, 0.10,0.01), respectively, while the frequency of the G allele was (0.94) and the frequency of the A allele was (0.06), and these percentages were identical to the allele ratios G and A in this study.

Table 4
Number and percentages of genotypes and alleles frequencies for 5-UTR of IGF-1 in local black goats

Mutation site (5-UTR)	(Genotype)	number	Percentage (%)	alleles	allele frequency
A182G	AA :Wild	0	0.00	A	0.06
	AG :Hetro mutant	2	0.09		
	GG :Homo mutant	32	0.91	G	0.94
total		34	1.00		
			Chi-square value (x2)	---	86.36**
			(**P<0.01)		

Relationship of the polymorphism of 5-UTR of IGF-1 gene to daily and total milk production

The study showed (Table 5) that the differences in 5-UTR were affected by highly significant differences ($p < 0.01$) in both genotypes GG and AG, as the GG genotype outperformed the daily milk production (DMY) and total milk production (TMY) (0.343 and 268.62 kg), respectively. The reason for the increase in the expression of IGF-1 growth factor from the region of synthetic genes may be due to a mutation in the carcinoma region by shifting the base A to G, and that individuals carrying the mutant allele GG caused an increase in the expression of IGF-1 during the estrous cycle, counterbalancing the individuals carrying one mutant allele (G). This increase in expression reflected positively on the daily and total milk production. As these results were consistent with several studies that recorded a significant superiority of genotypes in milk production, as (Di Stefano., 2017) indicated a significant superiority ($p < 0.05$) in 5-UTR for the daily milk production trait, as the composition outperformed The genotype GG (1.99 ± 0.52) on the GC and CC genotypes (1.64 ± 0.3 , 1.58 ± 0.05) respectively in Sarda sheep. (Sebastiano et al., 2020) indicated the presence of three genotypes for the 5-UTR region (CC, CG, GG), which were significantly ($P < 0.05$) superior to the individuals carrying the CC genotype in three stages of daily milk production (1800, 1580 (1990) compared to the carrier individuals). This was contrary to what was observed by (Deng et al., 2010) on two Chinese goats (Guanzhong and Xinony saanen), as there were no significant differences in both genotypes GG and AG for the same region 5-UTR. The AA genotype did not appear in the studied sample.

Table 5
Relationship of genetic polymorphism of 5-UTR of the IGF-1 gene in daily and total milk production

Influencing factors	number of animals	mean \pm SE	
		DMY	TMY
genotype	33		
GG	31	0.02 \pm 0.343 a	16.55 \pm 268.62 a
AG	2	0.01 \pm 0.213 b	9.12 \pm 167.02 b
Level of significance		**	**

The different letters within the column differ significantly from each other. DMY=daily milk production , TMY= total milk production ** (p< 0.01)

Relationship of the polymorphism of 5-UTR of IGF-1 gene to in growth traits

The results of the current study showed that there were non- significant differences for the trait of body weight at birth (BWT) and weight gain pre-weaning (WG). While there were highly significant differences (P < 0.01) in 5-UTR of body weight at weaning (WWT) according to the genotypes of IGF-1 gene, as the WWT of the chicks carrying the GG genotype was higher (14.39 kg) of individuals carrying the AG genotype (10.20 kg) (Table 6). The reason for the increase in the expression of growth factor IGF-1 from the region of the synthetic genes may be due to a mutation in the studied region by shifting the base A to G, and that individuals carrying the mutant allele GG caused an increase in the expression of IGF-1 during the estrous cycle offsetting the individuals carrying one mutant allele (G) and this The increase in expression was positively reflected on the traits of WWT because it is related to BWT, knowing that WWT is one of the most important economic traits in goat breeding flocks, as it is the main determinant of weight when marketing and also reflects the ability of the mother to produce milk.

The results of the study were in agreement with a number of studies in this field, as(Thomas et al., 2017) indicated in a study on two Indian goats that the insulin-like growth factor (IGF-1) gene is a member of the somatic axis and plays a major role in cell proliferation and differentiation in the growth of The fetus as well as postnatal growth, where it was significantly (P<0.05) superior to the individuals carrying the genotype GG (31.86 kg) in WWT compared to the individuals carrying the genotype AG (29.56 kg) in the Attappady Blak breed, and it did not show significant differences in WWT in Malabari breed. As well as what Bayraktar et al (2021) reported, where there were significant (p < 0.05) differences in WWT in the Iraqi Hamdania sheep breed, where the genotypes AA and BB gave a higher WWT (23.72 and 23.59 kg) respectively compared with the genotype AB (22.98) kg. While there were no significant differences in WG between the three genotypes (BB, AB, AA).

Table 6
Relationship of genetic polymorphism of 5-UTR of the IGF-1 gene to growth traits

Influencing factors	number of animals	mean \pm standard error		
		BWT	WWT	WG
GG	31	0.09 \pm 2.83 a	a 0.80 \pm 14.39	1.10 \pm 12.25 a
AG	2	0.50 \pm 2.50 a	b 1.10 \pm 10.20	0.60 \pm 7.70 a
Level of significance		ns	**	ns

Different letters within the column differ significantly from each other ** (p < 0.01), NS (non- significant).

References

- Al Jalili, Zuhair Fakhri, Jalal Elia Alqas. (1984). Sheep and goats production. Ministry of Higher Education and Scientific Research, Institution of Technical Institutes. Iraqi Republic.
- Alqas, Jalal Elia, Zuhair Fakhri Al Chalabi and Aziz, Daeb Ishaq. (1993). Basics of sheep and goat production and breeding, College of Agriculture, University of Baghdad, Dar Al-Kutub Press for Printing and Publishing.
- Al-Azzawi, Saleh Hassan. (2017). Genetic analysis of milk production and length of milk season for Shami goats under semi-intensive breeding conditions in central Iraq. Diyala District for Agricultural Sciences 9 (special issue): 16-24.
- Jayed, Talib Ahmed, Muntaha Yaqoub Yousef, Bashar Faleh Zughair, Jaafar Muhammad Owaid.(2011). Genetic morphology of the iron-to-blood protein transferrin (transferrin) in Arab sheep. Diyala Journal of Agricultural Sciences. 3(2): 69-76.
- Bayraktar, M., & Shoshin, O. 2021. Estimate of the Association of IGF-I and IGFALS Genes with Growth Traits in Hamdani Sheep. Brazilian Archives of Biology and Technology, 64.
- Deng, C., Ma, R., Yue, X., Lan, X., Chen, H., & Lei, C.(2010). Association of IGF-I gene polymorphisms with milk yield and body size in Chinese dairy goats. *Genetics and molecular biology*, 33, 266-270.
- Di Stefano, M. V.(2017). IGF-I and its role in dairy animals: investigations on Holstein Friesian cows and Sarda sheep. IGF-I and its role in dairy animals: investigations on Holstein Friesian cows and Sarda sheep, 78.
- Estany, J., Tor, M., Villalba, D., Bosch, L., Gallardo D., Jimenez, N. L., Altet, Noguera, J.L., Reixach, J., Amills, M. and Sanchez, A. (2007). Association of CA repeat polymorphism at intron 1 of insulin-like growth factor (IGF-I) gene with circulating IGF-1 concentration, growth and fatness in swine. *Physiol. Genomics*, 31:236-243.
- Ge, W., Davis, M.E., Hines, H.C., Irvin, K.M. and Simmen, R.C. (2001). Association of a genetic marker with blood serum insulin-like growth factor-I concentration and growth traits in Angus cattle. *J. Anim. Sci.*, 79: 1757-1762.

- Gootwine, E and Pollott, G. E. (2000). Factors affecting milk production in improved Awassi dairy ewes. *Animal Science*, 71: 607-615.
- Hashizume, T., Ohtsuki, K., and N. Matsumoto. (2000). Serum insulin-like growth factor-I concentrations increase during the estrous phase in goats. *Domestic Animal Endocrinology*. 18: 253-263.
- ICAR, (2004). International Committee for milk recording. Milk recording in sheep in ICAR member countries. ICAR, Rome.
- Jones, J. I., and D. R. Clemmons. (1995). Insulin-like growth factors and their binding proteins: biological actions, *Endocrine Review*. 16(1): 3-34.
- SAS. (2012). *Statistical Analysis System, User's Guide*. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
- Sebastiano, L., Consuelo, M. M., Luisa, P., Giovanni, C., Michella, N., & Vincenzo, C. (2020). Polymorphism of insulin-like growth factor 1 gene and its relationship with reproductive performances and milk yield in Sarda dairy sheep. *Veterinary and Animal Science*, 9, 100084.
- Todd, B. J., Merhi, Z. O., Shu, J., Etgen, A. M., and G. S. Neal-Perry. (2010). Hypothalamic insulin-like growth factor-1 receptors are necessary for hormone-dependent luteinizing hormone surges: Implications for female reproductive aging. *Endocrinology*. 151,449: 1356-1366.
- Naicy, T., Venkatachalapathy, T., Aravindakshan, T., Raghavan, K. C., Mini, M., & Shyama, K. (2017). Association of a novel single nucleotide polymorphism at the exon-2 of Insulin-Like Growth Factor 1 (IGF1) gene with phenotypic variants in goats. *Veterinarski arhiv*, 87(4), 457-472.
- Wathes, D. C., Reynolds, T. S., Robinson, R. S., and K. R. Stevenson. (1998). Role of the insulin-like growth factor system in uterine function and placental development in ruminants. *Journal of Dairy Science*. 81: 1778-1789.
- Yurnalis, A. and Putra, D.E. (2017). Polymorphism of Insulin-like Growth Factor 1 Gene (IGF1/TasI, IGF1/SnaBI, IGF1/RsaI) and the Association with Daily Gain of Pesisir Cattle Local Breed from West Sumatera, Indonesia. *Pakistan Journal of Biological Sciences*, 20: 210-216.
- Arnawa, I.K., Sapanca, P.L.Y., Martini, L.K.B., Udayana, I.G.B., Suryasa, W. (2019). Food security program towards community food consumption. *Journal of Advanced Research in Dynamical and Control Systems*, 11(2), 1198-1210.
- Gede Budasi, I. & Wayan Suryasa, I. (2021). The cultural view of North Bali community towards Ngidih marriage reflected from its lexicons. *Journal of Language and Linguistic Studies*, 17(3), 1484-1497
- Gandamayu, I. B. M., Antari, N. W. S., & Strisanti, I. A. S. (2022). The level of community compliance in implementing health protocols to prevent the spread of COVID-19. *International Journal of Health & Medical Sciences*, 5(2), 177-182. <https://doi.org/10.21744/ijhms.v5n2.1897>