

Prevalence of Mastitis and In-Vitro Antibiogram Study of the Mastitogens in Bhag-Nari Cattle

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Abstract

A study was designed to detect the mastitis and its mastitogens in Bhag-nai cows at district Naseerabad, Pakistan. Milk samples were collected from Bhag-nari cows. All information of milk samples (n=323) were collected randomly on the basis of designed performa (Annexure.1). Two to three strips of milk from each quarter were drawn on the floor surface to examine the presence of pus, blood clots, flakes and change in color. Strip cup test was applied to detect the clinical mastitis. Surf Field Mastitis Test (SFMT) was used for the detection of subclinical mastitis in Bhag-nari cows. Aseptic techniques were applied by using cotton swabs dipped into 70% ethanol to clean and disinfect teat end. Sterile tubes of 10ml capacity were used to collect the milk samples. The positive milk samples were kept immediately in an icebox cooler and transported to lab (CASVAB) in Quetta. Primarily each milk sample was cultured on Nutrient agar by spread out technique. Mannitol salt agar was used to culture the Staphylococcus aureus. Multiple streaking was applied to isolate the selected bacteria. On the basis of culture characters, microscopic morphology, staining method and biochemical tests bacterial isolates were identified. Prevalence of mastitis in Bhag-nari cattle in Naseerabad, Balochistan was 15.79%. Areas wise the prevalence of mastitis was 18.5%, 16.2%, 14.1% and 12.9% in DM Jamali, Chattar, Baba kot and Tamboo, respectively. Age wise prevalence in the study was 14.29%, 19.63%, 17.58% and 4.88% in age group of 3-5 years old, 6-8 years, 9-11 years and above 11 years, respectively. On the based on number of calving there was significant difference ($P<0.05$) among the various parity numbers. The animals milked once daily showed 17.06% mastitis as compared to 3.33% mastitis in animals daily milked more than once. There was significant difference ($P<0.05$). The prevalence of mastitis in well fed and under fed animals was 5.63% and 18.65%, respectively. Highly significance relation ($P<0.05$) was observed between the animals of satisfactory and none satisfactory udder hygiene with 6.94% and 33.64% prevalence. The most common bacterial isolates (staphylococcus aureus, streptococcus agalactiae and streptococcus dysgalactiae) were identified in the study. The most effective drugs against isolated bacteria were Ceftiofur, Oxytetracycline, chlortetracycline, Norfloxacin and Cephadrine.

Introduction

Contribution of Livestock sector is 11.8 % of Pakistan's GDP, with anticipated 43.562 billion liters of milk produced per annum. According to the economic survey of Pakistan, Pakistan is the fourth biggest nation in milk production in the world. Observing all the issues with respect to the dairy business of Pakistan, it is that the dairy business has capability of development and is essential from economic prospective (Anonymous, Economic survey of Pakistan 2014-2015).

Mastitis is a complex and multi-etiological condition, characterized as an inflammation of parenchyma of mammary organs. It causes physical and bacteriological changes in glandular tissues. It is due to three main considerations: irresistible operators, resistance of host and ecological variables. Although mastitis is a worldwide issue as it affects the animal health, nature of milking, decreases the milk production and causes

enormous losses to all countries (Sharma et al. 2012). From a monetary perspective mastitis is the most across the board irresistible disease in dairy cows and, the most harming one. Clinical and sub-clinical mastitis are the two types of the mastitis: Clinical mastitis shows physical changes in the udder, all signs of inflammation (pain, redness, swelling and with or without warmth in tainted mammary quarters), decrease in production of milk and effortlessly distinguished. Interestingly sub-clinical mastitis is more difficult to physically diagnose because the signs are not promptly evident (Kivaria, 2006), so it is difficult task to determine the sub-clinical mastitis in dairy cows in veterinary practice. The occurrence of clinical type is 15 to 40 less than the sub-clinical type (Seegers et al. 2003). The sub-clinical mastitis animals continuously affect the herd animals which remain until affected animal is separated from herd (Islam et al. 2011). Mastitis is detected in different levels as in an individual level, crowd level and bulk milk for the recognition of mastitis (Kivaria, 2006).

Materials and methods

Study area

The district Naseerabad is located at 27.38° latitude in North, longitude of East is 67.92° and elevation above the sea level is about 31 meter. The annual rainfall is 380mm. The temperature ranges from -2 to 25 centigrade in winter and 32 to 45 centigrade in summer. The district consists of 4 Tehsils and 24 Union Councils. The boundaries of the district in North reach to Jaffarabad, in East with Dera Bugti, Sibi and Bolan in the South and the West of the city is Larkana and Shahdadkot district of Sindh Province.

Source of Animals

The lactating Bhag-Nari cows reared from various small and large dairy farms in Naseerabad district included in this study to determine the prevalence of mastitis. A total of 323 Bhag-Nari cows were examined for the presence of clinical and subclinical mastitis. The sample size was calculated on the basis of expected prevalence of 30% using following formula as described by Thrusfield, (2005). Clinical mastitis was diagnosed on the basis of visible and palpable changes in milk and udder. These changes included watery milk with flakes, swollen, red, hot, and painful affected quarter (Radostits, et al. 2010). Stripcup test was also performed to diagnose clinical mastitis. On the other hand, subclinical mastitis was diagnosed using Surf Field Mastitis Test (SFMT) as described by Muhammad et al, (1995).

Stripcup Test (Procedure)

The strip cup test is a simple cow side test for determining the presence of clinical mastitis on the basis of presence of flakes in the milk. Briefly, first few streams of the foremilk from each of the quarters were squirted onto the strip cup (plastic paddle) having black background. The milk was visually examined for milk abnormalities such as flakes, clots, change in color, consistency. The milk samples having visible flakes and clots with watery appearance were considered as positive for clinical mastitis.

SURF FIELD MASTITIS TEST

Principle

Surf Field Mastitis Test uses 3% house hold detergent as reagent. When SFMT reagent mixed with milk containing somatic cell, the reagent causes destruction of cell wall and cell membrane of the somatic cell resulting into release nucleic acid into the solution. The nucleic acids of various somatic cell string each other to make gel.

Preparation of SFMT reagent

SFMT reagent was prepared in a glass beaker by dissolving 30 grams of household detergent (Surf Excel, Liver Brother, Pvt. Ltd, Pakistan) in 1000 mL of tap water. The solution was vigorously agitated for uniform mixing to make reagent. This SFMT reagent is stable for months at room temperature (Muhammad et al. 1995). After preparation reagent was transferred into dispensing bottle for field use.

Procedure

About 2-3mL milk sample from each quarter was collected directly from the teat in plastic paddle of black background having four receptacles. An equal amount of SFMT reagent (2-3mL) was added to each cup in the paddle from the dispensing bottle. Plastic paddle was then gently rotated in rotary action to thoroughly mix the contents. The test was read and reaction was visually scored.

Biochemical Tests and Antibiogram

Different biochemical tests were applied to identify the bacterial isolates and then antibiogram sensitivity test was applied to see the sensitivity of applied antibiotics.

Results and discussion

The different dairy species especially milking cows are very susceptible to mastitis. Due to lack of education on the occurrence and pathology of mastitis in cows remain non understandable. Surf Field Mastitis Test is used as indicator to find the presence of udder inflammation and to find out the infection. Strip cup test is applied to determine the clinical mastitis in dairy cows.

In the current study prevalence of mastitis in Bhag-nari cattle in Naseerabd, Balochistan was investigated. The prevalence of mastitis was 15.79% that is out of n=323, 51 cattle were showed positive by the diagnostic tests.

The prevalence of subclinical mastitis is 17.93% in India and Pakistan (Allore, 1993). Exotic breeds of cattle in KPK (Khyber Pakhtun Khwa) has higher prevalence of mastitis of 29.20% and Zebu cattle with 23.78% (Amjad et al., 2015). The prevalence of subclinical mastitis in Cholistani cattle of Pakistan is 21.96% monitored with CMT (A. Qayyum et al., 2016). The difference in prevalence of mastitis in cattle noted by various researcher may be due to difference in breed susceptibility, environmental condition, husbandry practices, diagnostic techniques and immune status of the animals.

In present study the prevalence of cattle was calculated on the basis of different areas of the District. It was 18.5%, 16.2%, 14.1% and 12.9% in DM Jamali, Chattar, Baba kot and Tamboo, respectively. There was none significant difference ($P>0.05$) among the different investigated areas.

Prevalence of mastitis in Tehsil Jand, Attock, Pinddighaib and Fateh Jang is 16.66%, 13.33%, 11.67% and 13.33%, respectively (Bachaya et al. 2005). The different areas of the district are almost having similar environmental conditions and same nutrients are available for the animals throughout.

Age wise prevalence in the study was 14.29%, 19.63%, 17.58% and 4.88% in age group of 3-5 years old, 6-8 years, 9-11 years and above 11 years, respectively.

The results are similar with A. Qayyum et al. (2016) who noted the highest prevalence in age group of animals with 6-9 years old. The researcher noticed 3-6 years, 6.1-9 years, 9.1-12 years and >12 years with 19.55%, 23%, 17.7%, and 32.4%, respectively. The results of the current study are supported by the results of Hussain et al. (2013) who reported none significant ($P>0.05$) relation of age group with mastitis.

Another factor of prevalence based on number of calving was studied in the study. There was significant difference ($P<0.05$) among the various parity numbers. Highest prevalence was observed in the 4th calving cattle (25%). A. Qayyum et al. (2016) also noted significance difference in mastitis prevalence on the basis of

parity number. The reason behind the factor may be due to relaxed sphincter muscles of teat orifice with passage of time. The factor may be due to vitamin and selenium deficiency with age.

Evaluation of Bhag-Nari cattle mastitis on the basis of frequencies of milking was also determined in the study. The animals milked once daily was showed 17.06% mastitis as compared to 3.33% mastitis in animals daily milked more than once. There was significant difference ($P < 0.05$) between the factor of milking frequencies in the study. Mastitogenic pathogens invade the teat and reside in the udder. The multiplication of organism increases with increase milk retention time in the udder. Milk is best growing nutrient for bacteria. The longer milk and the invaded organism retain in the udder the more; prevalently leads to inflammation of the udder parenchyma.

Table 4.1. Overall prevalence of mastitis in Bhag-Nari cows in Naseerabad district.

No. examined Cows	No. of cows positive	Prevalence %	P-value
323	51	15.789	0.000

Table 4.2. Prevalence of subclinical and clinical mastitis in Bhag-Nari cows in Naseerabad district.

No. examined	No. positive for subclinical mastitis	Prevalence (%)	No. positive for clinical mastitis	Prevalence (%)
323	44	13.622	7	2.17

Table 4.3. Area wise prevalence of mastitis in Bhag-Nari cows in Naseerabad district.

Area	No. examined	No. positive	Prevalence (%)	P-value
DM Jamali	97	18	18.556	0.770
Chattar	86	14	16.279	
Baba kot	78	11	14.102	
Tamboo	62	8	12.90	

Table 4.4. Age wise prevalence of mastitis in Bhag-Nari cows in Naseerabad district.

Age group	No. examined	No. positive	Prevalence (%)	P-value
3-5 years	84	12	14.29	0.156
6-8 years	107	21	19.63	
9-11 years	91	16	17.58	
Above 11 years	41	2	4.88	

Table 4.5. Parity prevalence of mastitis in Bhag-Nari cows in Naseerabad district.

Parity no.	No. examined	No. positive	Prevalence (%)	P-value
I.	30	7	23.33	0.044
II.	72	5	6.94	
III.	105	17	16.190	
IV.	60	15	25	
>IV	56	7	12.5	

Table 4.6. Prevalence of mastitis on the basis of milking frequency in Bhag-Nari cows in Naseerabad district

Milking frequency	No. examined	No. positive	Prevalence (%)	P-value
Once	293	50	17.06	0.049
More than once	30	1	3.33	

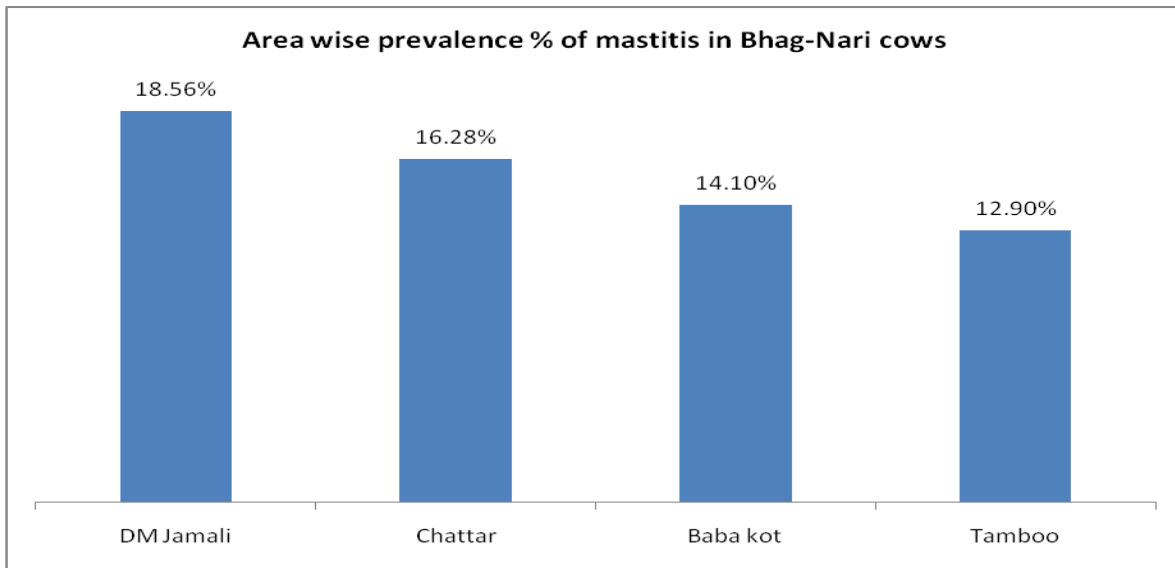


Figure 4.1. Area wise prevalence % of mastitis in Bhag-Nari cows.

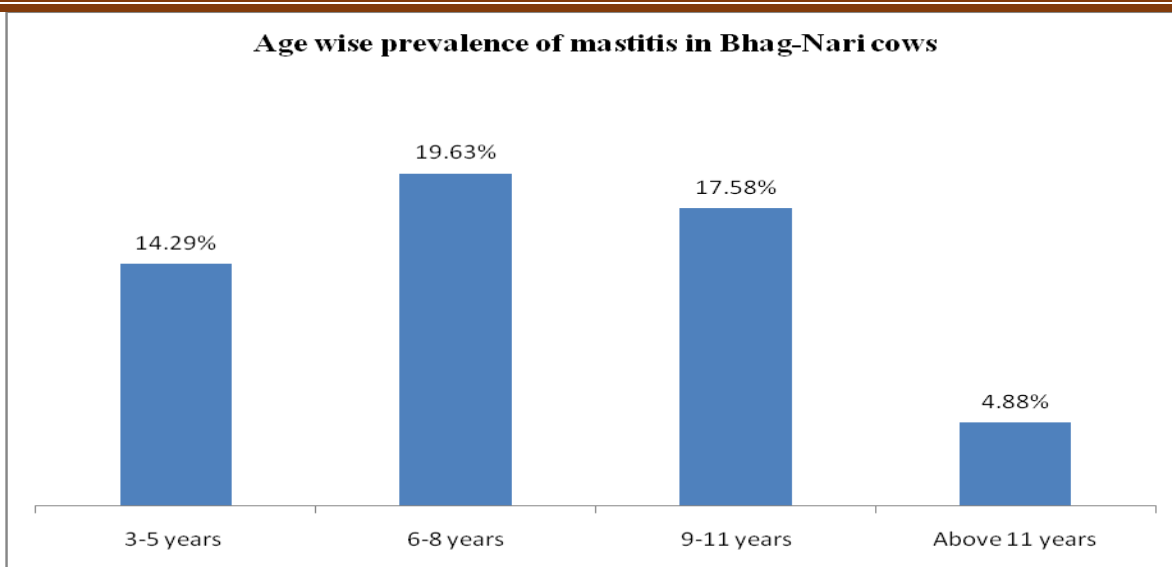


Figure 4.2. Age wise prevalence of mastitis in Bhag-Nari cows

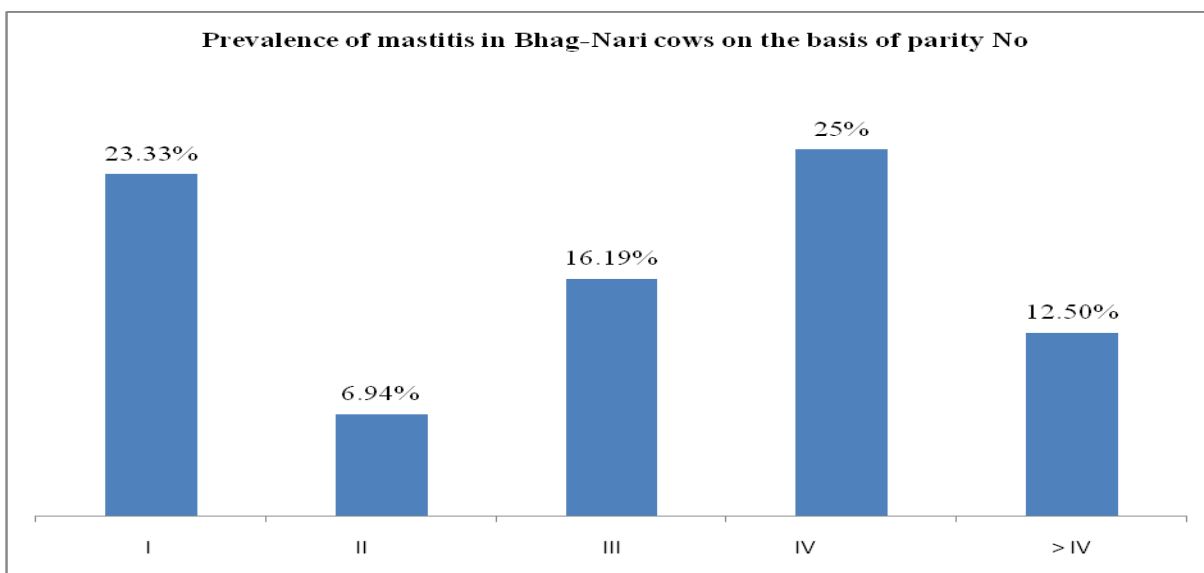


Figure 4.3. Prevalence of mastitis in Bhag-Nari cows on the basis of parity number.

Table 4.7. Prevalence of mastitis on the basis of teat dipping performance in Bhag-Nari cows in Naseerabad district.

Teat dipping	No. examined	No. positive	Prevalence (%)	P-value
Performed	39	2	5.13	0.034
Not performed	284	49	17.25	

Table 4.8. Prevalence of mastitis in Bhag-Nari cows on the basis of quantity of nutrition in Naseerabad district.

Feeding	No. examined	No. positive	Prevalence (%)	P-value
Well fed	71	4	5.63	0.006
Under fed	252	47	18.65	

Table 4.9. Prevalence of mastitis in Bhag-Nari cows on the basis of udder hygiene in Naseerabad district.

Udder hygiene condition	No. examined	No. positive	Prevalence (%)	P-value
Satisfactory	216	15	6.94	0.000
Non-satisfactory	107	36	33.64	

Prevalence of Bacteria:

According to the cultural bacterial isolates the most dominant isolate was *Staphylococcus aureus* (50.98%) followed by *Streptococcus agalactiae* (29.41) and *Streptococcus dysagalctiea* (19.60).The percentage of bacterial isolates are summarized in table 4.16. The results of chi-square showed significant difference among the isolates as p-value was less than 0.05.

Table 4.16. Prevalence of Bacterial isolates.

Organism	Number	Percentage
<i>Staphylococcus aureus</i>	26	50.98
<i>Streptococcus agalactiae</i>	15	29.41
<i>Streptococcus dysagalactiae</i>	10	19.60

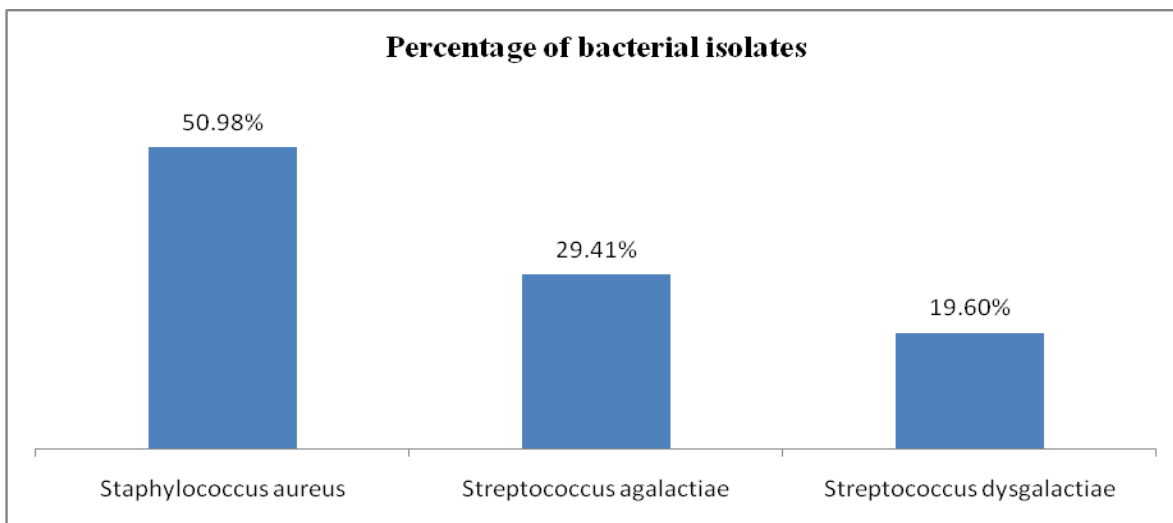


Figure 4.4. Percentage of bacterial Isolates.**Antibiogram Sensitivity Testing**

The antibiogram sensitivity tests showed that the *Staphylococcus aureus* was sensitive to Ceftiofur, Oxytetracycline, chlortetracycline and Cephadrine. It showed intermediate activity against Oxytetracycline, Ciprofloxacin and Amoxicillin. It showed resistive to penicillin. *Streptococcus agalactiae* was sensitive to Ceftiofur, Oxytetracycline, chlortetracycline Cephadrine and Norfloxacin. It showed intermediate activity against Amoxicillin, Ciprofloxacin and Penicillin. The antibiogram sensitive activity of *Streptococcus dysagalctiea* was almost similar with *Streptococcus agalactiae*. Table- 4.17 showed the antibiogram sensitivity of isolates against different antibiotics. The chi square result indicated significant difference among antimicrobials against different isolates as p- value less than 0.05.

Table 4.17. Antibiotic disc sensitivity of bacterial isolates.

Organism	Sample Numbers	Applied Antibiotic Discs							
		PEN	AMX	OTC	CTC	NOR	CIP	CEPH	CEF
<i>Staphylococcus aureus</i>	1	I	I	I	S	S	S	S	S
	2	I	I	S	S	S	S	S	S
	3	R	I	S	S	S	I	S	S
	4	R	I	R	I	S	I	I	S
	5	R	R	S	S	S	I	I	I
<i>Streptococcus agalactiae</i>	1	R	I	I	S	S	S	S	S
	2	R	I	I	S	I	R	I	S
	3	I	I	S	S	S	S	S	S
	4	I	I	S	S	S	I	S	S
	5	I	I	S	S	S	I	S	S
<i>Streptococcus dysagalctiea</i>	1	I	I	S	S	S	S	S	S
	2	R	R	S	S	S	I	S	S
	3	R	I	S	S	I	I	S	S
	4	R	I	I	S	S	I	S	I
	5	R	I	I	I	S	I	S	S

PEN=Penicillin, AMX= Amoxicillin, OTC= Oxytetracycline, CTC= Chlortetracycline, NOR = Norfloxacin, CIP = Ciprofloxacin, CEPH= Cephadrine, CEF= Ceftiofur.

Conclusion

It was concluded from the current study that mastitis is prevalent in Bhag-Nari cattle in district Naseerabad, Balochistan. The most common pathogens involved in Bhag-Nari cattle mastitis are the higher ratio of *Staphylococcus aureus* followed by *Streptococcus agalactia* and *Streptococcus dysagalctia*. The sensitivity order of the mastitis causing bacteria to the antibiotics in antibiogram study are Ceftiofur, Oxytetracycline, chlortetracycline and Cephradine from most sensitive to lesser. Whereas intermediately sensitive to Oxytetracycline, Ciprofloxacin and Amoxicillin and resistance to Penicillin group.

Suggestion

Bhag-Nari is the only dual purpose cattle breed of Balochistan. The cattle have developed resistance to harsh environmental conditions of its home tract through centuries. The production potential (beef, milk) of the breed may be assessed and practical scientific approaches should be developed to improve the animal and facilitate the farmer.

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