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Evaluation of *in-vivo* efficacy of MESTICHECK for diagnosis of clinical and subclinical mastitis in milch animals

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Abstract
Mastitis is one of the major challenges faced by veterinarians, government and policy makers working on socio-economic upliftment of rural livestock owners. Undiagnosed and unattended cases of subclinical mastitis tend to convert into clinical mastitis which affects the quality of milk and forces livestock owners to suffer from economic losses. Therefore, there is a need to evaluate existing status of subclinical mastitis in milch animals. Various diagnostic modalities are available to diagnose mastitis; however, a relatively easy, quick and cost-effective test should be available at field level for early detection of subclinical mastitis. Such tests are always required to be evaluated for their clinical applicability. The present investigation was conducted to evaluate *in-vivo* efficacy of MESTICHECK component of Mulchize farm hygiene kit for diagnosis of clinical and subclinical mastitis in milch animals at Postgraduate Institute of Veterinary Education & Research (PGIVER), Kamdhenu University, Rajpur (Nava), Himmatnagar during August-2020 to January-2021. The result of MESTICHECK was compared with the results of 08 other diagnostic modalities established to diagnose mastitis in cases registered at Veterinary Hospital. Based on the study, it is concluded that MESTICHECK has observable diagnostic value and feasibility of using at field level. Further evaluation and use of MESTICHECK is encouraged through extensive investigations including greater number of cases.

Keywords: mastitis, diagnosis, MESTICHECK, Mulchize kit, *in-vivo*

Introduction
Mastitis is a disease of udder/mammary gland of milch animals (e.g., cattle, buffalo, goat). It has a wide range of etiological factors such as infectious organisms (e.g., bacteria, fungus, yeast etc.), traumatic conditions (e.g., injury to udder/teat), toxic factors etc. Some of the bacterial and fungal pathogens responsible for causing mastitis include *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysagalactiae*, *Streptococcus uberis*, *Streptococcus bovis*, *Escherichia coli*, *Streptococcus zooepidermicus*, *Streptococcus faecalis*, *Streptococcus pyogenes*, *Corynebacterium pyogenes* ('Summer mastitis'), *Pasteurella multocida*, *Pseudomonas pyocyaneus*, *Klebsiella pneumonia* ('Peracute Bovine Mastitis'), *Serratia* spp., *Proteus* spp., *Enterobacter* spp., *Leptospira pomona*, *Mycobacterium bovis*, *Brucella abortus*, *Mycoplasma bovis*, *Mycoplasma bovigenetelium*, *Aspergillus fumigatus*, *Aspergillus modulus*, *Candida* spp., *Cryptococcus neoformans* etc [1,2,3]. Bacterial mastitis is most commonly reported and studied throughout the world. Moreover, most of the clinical or subclinical cases of mastitis are treated with antibiotics and anti-inflammatory drugs under field conditions believing the bacterial pathogens as the common etiology. This haphazard and inappropriate use of antibiotics has also increased antibiotic resistance in field [4]. Therefore, early and specific diagnosis of mastitis must be performed to get appropriate results and to prevent antibiotic resistance in field.

Mastitis is broadly classified as Clinical Mastitis (CM) and Subclinical Mastitis (SCM) based on clinical presentation. Cases of CM are more frequently encountered at veterinary hospitals as compared to SCM. This could be due to the fact that SCM often goes unnoticed at farm level and gets converted to CM because of lack of timely treatment. Various diagnostic tools are available for diagnosis of CM and SCM where California Mastitis Test (CMT) is considered as one of the easiest and quickest method. Many commercial CMT kits are available in market, but it is perceived that livestock owners do not have enough exposure or

availability of CMT. Additionally, CMT is also not used at many hospitals on regular basis. Therefore, there is a need to evaluate some CMT concept-based diagnostic methods for their feasibility of using at field level. With a similar vision, an agency Bharat Biogas Pvt. Ltd., Ahmedabad (Gujarat) had provided 'Mulchize' animal farm hygiene kit which contains different components including a CMT concept MESTICHECK kit for diagnosis of mastitis in milch animals. The present investigation was conducted to evaluate *in-vivo* efficacy and feasibility of using MESTICHECK at field level by comparing its results with other established diagnostic modalities for mastitis. The present paper highlights significant observations of the study.

Materials & Methods

The present study was undertaken to evaluate the components of an animal farm hygiene kit 'Mulchize' commercially developed to encourage clean milk production at organized or unorganized milch animal farms. The Mulchize kit contains a mastitis diagnosis test kit based on the concept of CMT. This component is termed as 'MESTICHECK' and it is provided as a diagnostic reagent along with four-well paddle (Figure-1). Characteristic 'Gel formation' when the reagent is added to the milk sample was required to be observed and interpreted as a 'Positive' test for mastitis [for both, CM & SCM]. On the other hand, an absence of gel formation was interpreted as a 'Negative' test. The MESTICHECK was added as an additional diagnostic modality to diagnose CM in 07 cases (05 buffaloes + 02 cattle) and SCM in 03 cases (03 buffaloes) registered at Veterinary Hospital functional under the Postgraduate Institute of Veterinary Education & Research (PGIVER), Kamdhenu University, Rajpur (Nava), Himmatnagar during August-2020 to January-2021. The cases of CM and SCM were included irrespective of age, breed and type of milch animals (*i.e.*, cattle or buffalo) because the results were required to be interpreted on the basis of characteristic 'Gel formation' after using MESTICHECK. All the cases of CM and SCM were subjected to anamnesis and clinical examination. The pH of fresh milk samples from each udder quarter/teat was evaluated using a pH paper and pH meter. The Electric Conductivity (EC) of fresh milk samples from each udder quarter/teat was also measured by use of EC meter/machine (Draminski®). Fresh milk samples were also subjected to CMT using a commercially available and standardized kit (DeLaval). This step was specifically included to observe and compare the 'Gel formation' while using a commercially available kit and MESTICHECK on a common sample (Figure-2).

Fig 1: MESTICHEK & four-well paddle provided within the Mulchize kit

Fig 2: Commercially available CMT reagent (Left) and MESTICHECK reagent (Right)

In order to correlate the diagnostic results, the fresh milk samples from animals diagnosed with CM and SCM were also collected in sterile vials/containers (Eppendorf) for bacteriological cultural isolation and identification of bacteria at Central Diagnostic Laboratory, Polytechnic in Animal Husbandry (PAH), Kamdhenu University, Rajpur (Nava), Himmatnagar. The Bromothymol Blue (BTB) test, White Side Test (WST) and Catalase test were also performed for diagnosis of mastitis.

Results

A total of 07 cases were diagnosed with CM while 03 cases were diagnosed with SCM using various diagnostic modalities during the study period. The characteristic 'Gel formation' (Figure-3) while using MESTICHECK in cases of CM and SCM was consistent with the positive results of all other diagnostic methods as shown in Table-1 and Table-2. The test is relatively easy and quick to perform.

Table 1: Comparative evaluation of MESTICHECK with other diagnostic methods in 07 cases of Clinical Mastitis (CM)

Diagnostic Tests for CM								
Case No.	**pH Paper**	**pH Meter**	**BTB Test**	**CMT-DeLaval**	**Culture**	**Electric Conductivity**	**Bacterial Id**	**MESTICHECK (Gel formation)**
Case-1	>8	8.2	Positive	Positive	Positive	RF: 210 RH: 200 LF: 190 LH: 180	*Streptococcus* spp. *Staphylococcus* spp.	Positive
Case-2	>8	8.1	Positive	Positive	Positive	RF: 220 RH: 230 LF: 190 LH: 200	*Streptococcus* spp. *Staphylococcus* spp.	Positive
Case-3	7 to 8	7.9	Positive	Positive	Positive	RF: 220 RH: 230 LF: 240 LH: 210	*Streptococcus* spp.	Positive

Case-4	7 to 8	7.8	Positive	Positive	Positive	RF: 220 RH: 210 LF: 240 LH: 190	*Streptococcus* spp.	Positive
Case-5	>8	8.5	Positive	Positive	Positive	RF: 200 RH: 190 LF: 180 LH: 210	*Streptococcus* spp. *Staphylococcus* spp.	Positive
Case-6	>8	8	Positive	Positive	Positive	RF: 200 RH: 190 LF: 190 LH: 210	*Streptococcus* spp. *Staphylococcus* spp. *Escherichia coli*	Positive
Case-7	7 to 8	7.6	Positive	Positive	Positive	RF: 210 RH: 190 LF: 200 LH: 210	*Streptococcus* spp.	Positive

Table 2: Comparative evaluation of MESTICHECK with other diagnostic methods in 03 cases of Subclinical Mastitis (03 cases)

Diagnostic Tests for SCM								
Case No.	**pH Paper**	**pH Meter**	**BTB Test**	**CMT-DeLaval**	**Culture**	**Electric Conductivity**	**Bacterial Id**	**MESTICHECK (Gel formation)**
Case-1	7 to 8 (LH)	7.9	Positive	Positive	Positive	RF: 310 RH: 320 LF: 330 LH: 240	*Streptococcus* spp.	Positive
Case-2	7 to 8 (RH)	8	Positive	Positive	Positive	RF: 290 RH: 230 LF: 310 LH: 340	*Streptococcus* spp.	Positive
Case-3	7 to 8 (RF)	7.9	Positive	Positive	Positive	RF: 220 RH: 300 LF: 330 LH: 360	*Streptococcus* spp.	Positive

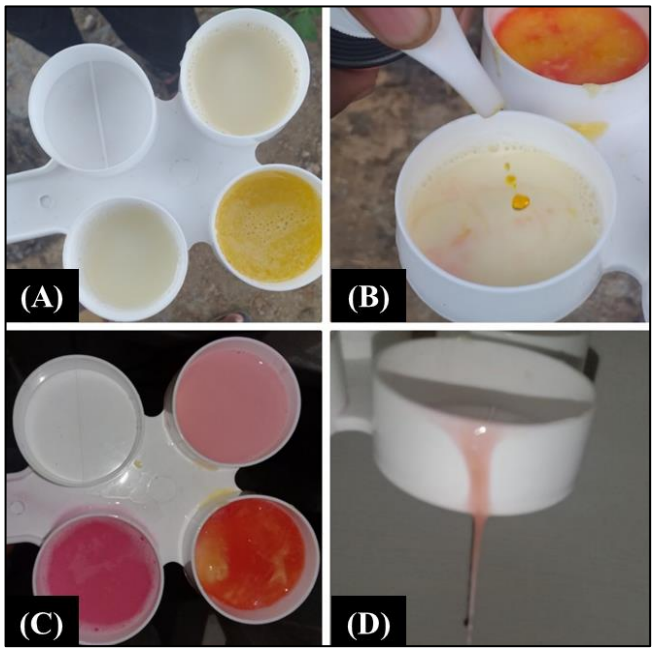

Fig 3: (A) Milk samples collected in four-well paddle in case of CM; (B) adding MESTICHECK reagent; (C) Mixing & gentle rotation of paddle; (D) Characteristic 'Gel formation' indicating a test 'Positive' for mastitis

Discussion

India has tremendous population of milch animals (e.g., cattle, buffalo, sheep, goat, camel etc.) and the country's milk production is higher as compared to many other countries. Despite topping the charts in terms of quantity of milk production, the quality of milk is generally questioned when there are opportunities for export of milk and milk products to other countries. The quality of milk is affected by factors such as presence of high bacterial load, higher somatic cell count, presence of adulterants, presence of antimicrobial drugs etc.

Currently, the government is planning to increase the income of farmers and livestock owners where milk and milk products also play a key role for their economic upliftment. Livestock owners generally suffer from economic losses due to depleted milk quality resulting in reduced or no market value for their milk. The quality of milk has direct relation with udder health. Out of all udder/mammary gland affections, mastitis is an inflammatory condition which is caused by many infectious and some non-infectious etiological factors. Mastitic milk is often found to have increased somatic cell count and increased pH favoring growth as well as multiplication of bacteria. Sometimes, mastitic milk also has a detectable presence of antibiotics which are used for treatment.

In past, the government and many academic institutes have undertaken and completed many target-specific research projects on mastitis. Outcomes of such projects have resulted in advancement in diagnostics and therapeutics. Out of all, the CMT is a diagnostic method used to diagnose SCM and it can be used as an additional diagnostic modality in case of CM. The cost of CMT is less as compared to other advanced techniques. CMT is relatively easy and quick to perform. It is perceived that many veterinary practitioners do not use CMT during their routine clinical practice. Moreover, many livestock owners (including those with single animal, marginal farmers with livestock and some commercial milch animal farm owners) also do not have enough exposure and availability of CMT. Various commercial CMT kits are

available in India and many research institutes as well as companies are also manufacturing kits for diagnosis of mastitis.

Similarly, a commercial farm hygiene kit Mulchize provided by Bharat Biogas Pvt. Ltd., Ahmedabad to promote clean milk production encompasses various components including MESTICHECK for diagnosis of mastitis. MESTICHECK component showed promising results when compared with results of established diagnostic methodologies of CM and SCM. Similar or extensive studies have not been conducted previously on MESTICHECK in the region.

Based on the observations recorded in the study, it is concluded that MESTICHECK has diagnostic value and feasibility of using at field level. Noticeably, MESTICHECK was provided as a component of Mulchize kit which also has a detergent (to wash milking utensils), a towel (to clean udder), a teat-dipping agent, a teat-dipping cup, a water disinfecting tablet, hoof spray, hoof knife and an alcohol-free sanitizer. The concept of encompassing such components in a single kit will encourage the livestock owners (*i.e.*, end-users) to adopt suitable hygienic practices for clean milk production. Additionally, MESTICHECK present in the kit would also help farmers/livestock owners for early detection of SCM in milch animals at field level, prevent SCM converting into CM, facilitate timely provision of treatment and reduce treatment cost.

Conclusion

MESTICHECK is relatively easy and quick to perform. It has shown promising results for diagnosis of clinical as well as subclinical mastitis in milch animals when compared with the results of established diagnostic modalities in this study. This observation also suggests and encourages use of MESTICHECK as an on-field or in-house diagnostic test for mastitis after conducting an extensive investigation on greater number of cases in future.

Conflict of Interest

Authors declare no conflict of interest with regards to funding. The project was approved by the Hon'ble Vice Chancellor, Kamdhenu University, Gandhinagar. The project was funded by Bharat Biogas Pvt. Ltd., Ahmedabad (Gujarat) which also provided the kits. Necessary permissions were received before initiating the work. All M.V.Sc. scholars were actively involved in sampling and laboratory procedures.

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