

Studies on Early Detection of Sub Clinical Mastitis and Its Antibiogram in Lactating Dairy Cattle

P. Revathi^{1*}, M. Vijaya Bharathi², A. Elango³, Mitali Inder Bhambhani⁴, B. Nagarajan⁵, D. Bala Subramanyam⁶

¹Ph. D. scholar, Department of Veterinary Preventive Medicine, Madras Veterinary College, Chennai, INDIA

²Associate Professor, Post Graduate Research Institute in Animal Sciences, Kattupakkam, INDIA

³Professor, Post Graduate Research Institute in Animal Sciences, Kattupakkam, Kancheepuram District, INDIA

⁴M. V. Sc. Scholar, Department of Veterinary Preventive Medicine, Madras Veterinary College, Chennai, INDIA

⁵Professor & Head, Dept. of Veterinary Preventive Medicine, Madras Veterinary College, Chennai, INDIA

⁶Professor & Head, Post Graduate Research Institute in Animal Sciences, Kattupakkam, Kancheepuram, INDIA

*Corresponding Author: pagadalarevathi@gmail.com

How to cite this paper:

Pagadala, R., Mangalanathan, V., A, E., Bhambhani, M., B, N., & D, B. (2020). Studies on early detection of sub clinical mastitis and its antibiogram in lactating dairy cattle. *International Journal of Livestock Research*, 10(9), 210-216. doi: <http://dx.doi.org/10.5455/ijlr.20200528110737>

Received : May 28, 2020
Accepted : Jul 30, 2020
Published : Sep 30, 2020

Copyright © Pagadala *et al.*, 2020

This work is licensed under the Creative Commons Attribution International License (CC BY 4.0). <http://creativecommons.org/licenses/by/4.0/>



Open Access

Abstract

A study was conducted in an organized farm to know the prevalence status of subclinical mastitis. Screening of animals for subclinical cases was done by using TANUCHEK-SCC kit developed by TRPVB, TANUVAS Chennai. A total of 167 quarters from 46 lactating dairy cattle were screened for mastitis. Out of these, 42 samples were found positive for subclinical mastitis and none of the samples were found positive for clinical mastitis with visible changes. The positive samples for subclinical mastitis were further subjected for invitro antibiotic sensitivity test using antibiotic discs like ceftizoxime, amoxicillin/sulbactam, ceftriaxone, enrofloxacin, cefalexin, gentamicin and cefoperazone/tazobactam. Antibiogram studies revealed enrofloxacin (100 per cent) is the most sensitive drug followed by gentamicin (97.6 per cent), amoxicillin/sulbactam (90.4 per cent), cefalexin (76.2 per cent), ceftriaxone (59.5 per cent), cefoperazone/tazobactam (38.1 per cent) and ceftizoxime (35.7 per cent). In the study, Enrofloxacin was the sensitive antibiotic found against subclinical cases.

Keywords: Antibiogram, Subclinical mastitis, TANUCHEK Somatic cell count test

Introduction

Mastitis is an inflammation of the mammary gland which together with physical, chemical and microbiological changes, is characterized by an increase in the number of somatic cells in the milk and by pathological changes in the mammary tissue (Giannechini *et al.*, 2002). Different pathogens can cause chronic, subacute, acute, peracute and subclinical forms of the disease (Radostits *et al.*, 2007). In clinical mastitis all the five cardinal signs (redness, swelling, heat, pain and loss of milk production) of udder inflammation are present (Bachaya *et al.*, 2011). There are no visible abnormalities of the milk or udder instead there is a high somatic cell count in SubClinical Mastitis (SCM) (Radostits *et al.*, 2007). Subclinical mastitis was defined as when mammary glands without clinical abnormalities giving apparently normal milk but was bacteriologically positive and with positive CMT (Stefanakis *et al.*, 1995).

SCM is 3–40 times more common than clinical mastitis and causes the greatest overall losses in most dairy herds (Bachaya *et al.*, 2011). Inflammation of mammary gland is directly accompanied by an increase of somatic cell count (SCC) in milk (Rodriguez *et al.*, 2000). Therefore, many reports have considered SCC as a significant marker for sub - clinical mastitis (Durr *et al.*, 2008).

The bacteria causing mastitis, rapidly acquire resistance due to frequent and indiscriminate use of antibacterials in treatment, which has been growing concern worldwide (WHO, 2000). In order to control mastitis antimicrobial sensitivity test is recommended by OIE. Therefore, establishing an antibiogram of pathogens is very important from the clinical and economic points of view. The susceptibility pattern of these microbes to the antibacterial drugs in use is constantly changing and susceptibility of the causing organisms is necessary for the success treatment.

Based on the above facts, the present study was focused with the following objectives.

Objectives

1. To detect the sub clinical mastitis in lactating dairy cross bred cattle from an organized farm by using TANUCHEK- SCC kit.
2. To study the *in-vitro* antibiotic sensitivity pattern of positive cases against different antibiotics.

Materials and Methods

Study Area

This study was conducted in 167 samples from 46 lactating dairy cattle at Cattle and buffalo breeding unit, Kattupakkam, Kancheepuram district affiliated to Tamil Nadu Veterinary and animal sciences university, Chennai.

1. TANUCHECK- SCC kit procured from TRPVB, Chennai.
2. B. O. D incubator (Manish scientific instruments company, Chennai), laminar air flow (Klenzaid's gradvel laminar flow clean air work station, Bombay)
3. Antibiotic discs ceftizoxime (30µg), amoxicillin/ sulbactam (30/15µg), ceftriaxone (30µg), enrofloxacin (10µg), cefalexin (30µg), gentamicin (10µg) and cefoperazone/ tazobactam (75/10µg) from M/s Hi Media Laboratories Ltd., Mumbai.
4. Nutrient broth and Mueller- Hinton agar from M/s Hi Media Laboratories Ltd., Mumbai.

1) TANUCHECK- SCC test

In the present study, subclinical mastitis cases were screened based on the somatic cell count by using TANUCHEK- SCC kit. This test was performed using TANUCHEK- somatic cell count kit developed by Translational research platform for veterinary biologicals (TRPVB), Tamil Nadu Veterinary and animal sciences university, Chennai. Microfuge tubes provided in the kit were labelled. One drop of substrate solution and three drops of enhancer

solution were added and mixed well by tapping the tubes gently. The teats were washed properly prior to collection and first few strippings were discarded. Milk samples were collected in a clean container and one drop of milk was added to that tube with the help of pasteur pipette and mixed well. The tubes were incubated at room temperature for 30 minutes. The development of colour was compared with the colour card provided on the kit. The number of somatic cells per ml of milk was obtained by matching the colour card multiplied by 1000 (100x 1000 cells, 300x 1000 cells, 500x 1000 cells, 700x 1000 cells, 900x 1000 cells) as per manufacturers guide lines. Somatic cell count of 5,00,000 to 7,00,000 cells per ml of milk were considered as subclinical mastitis cases.

Antibiogram Studies

The milk samples that were found positive for subclinical mastitis were collected aseptically and inoculated into nutrient broth and incubated at 37°C for 4-6 hours. Later, invitro antibiotic sensitivity test was done as per the guidelines of National Committee for Clinical Laboratory Standards (NCCLS). It was conducted on Mueller- Hinton agar (M/s Hi Media Laboratories Ltd., Mumbai) plates against commonly used antibiotics in the field like ceftizoxime (30µg), amoxicillin/ sulbactam (30/15µg), ceftriaxone (30µg), enrofloxacin (10µg), cefalexin (30µg), gentamicin (10µg) and cefoperazone/ tazobactam (75/10µg) as per the method of Bauer *et al.*, (1966).The zone of inhibition was compared with the standards provided by the manufacturer to evaluate the sensitivity and resistance pattern of particular antibiotic.

Results and Discussion

TANUCHECK-SCC test

In the present study, out of 167 quarters tested, 42 samples (25.15 per cent) were found positive for subclinical mastitis. Clinical mastitis cases were not found in the study. None of the milk samples were showed any physical abnormalities like colour, odour, presence of clots and consistency. According to international dairy federation criteria, 25.14 per cent quarters were suffering from sub clinical mastitis having somatic cell count of more than 5,00,000 per ml of milk. Among 46 animals screened, eight animals (17.4 per cent) were affected with more than one quarter. Percentage of somatic cell count among the screened animals was displayed in fig.1

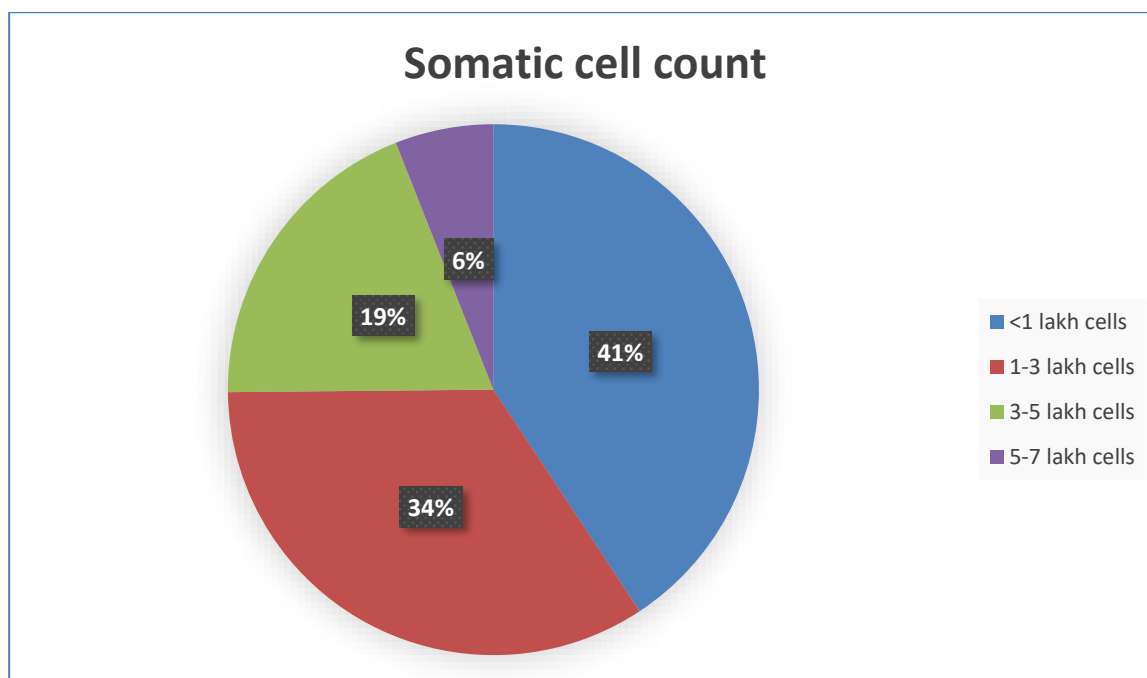


Figure 1: Displaying somatic cell count in the screened animals



Figure 2: Comparison of colour development with the colour card

Antibiogram Studies

Antibiotic sensitivity test of 42 milk samples revealed different sensitivity patterns which were expressed in percentage. enrofloxacin (100 per cent) is the most sensitive drug followed by gentamicin (97.6 per cent), amoxicillin/ sulbactam (90.4 per cent), cefalexin (76.2 per cent), ceftriaxone (59.5 per cent), cefoperazone/ tazobactum (38.1 per cent %) and ceftizoxime (35.7 per cent). The percentage of sensitivity pattern was displayed in table-1 and figure-3.

Table-1: Percentage of Sensitivity pattern

S. No.	Antibiotic	Sensitivity (%)	Intermediate (%)	Resistance (%)
1	Ceftizoxime (CZX)	35.7	2.3	62
2	Amoxicillin/ Sulbactam (AMS)	90.4	9.5	0
3	Ceftriaxone (CTR)	59.5	9.5	31
4	Enrofloxacin (EX)	100	0	0
5	Cefalexin (CN)	76.2	0	23.8
6	Gentamicin (GEN)	97.6	2.3	0
7	Cefoperazone/ Tazobactum (CST)	38.1	35.7	26.1

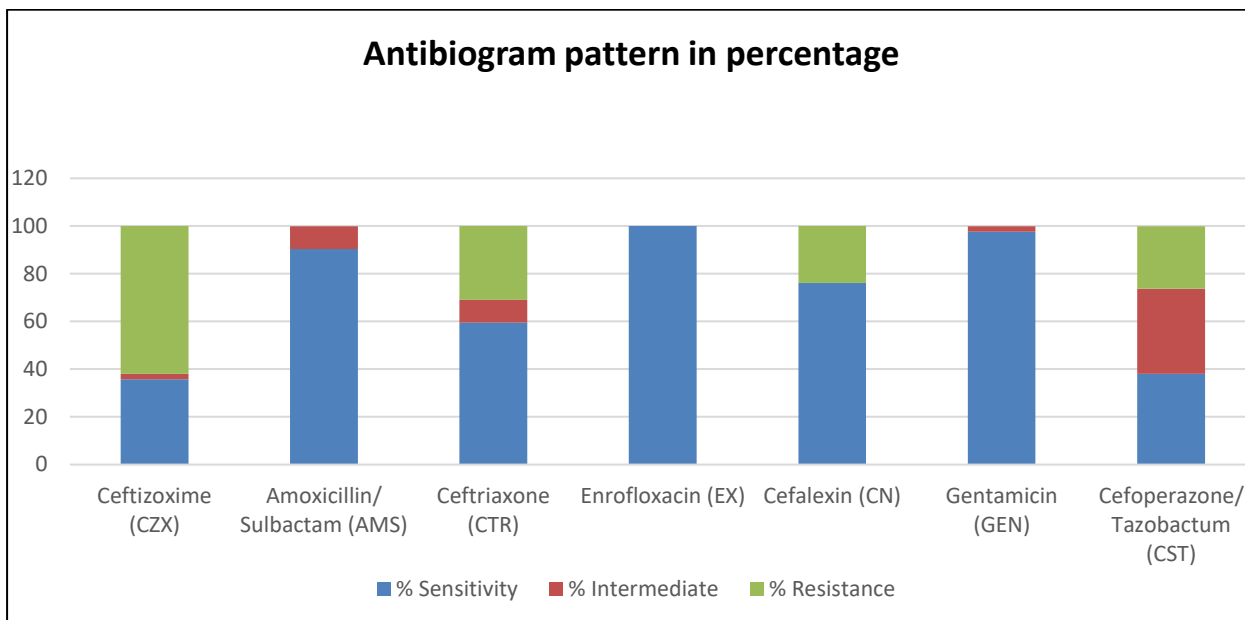


Figure 3:????

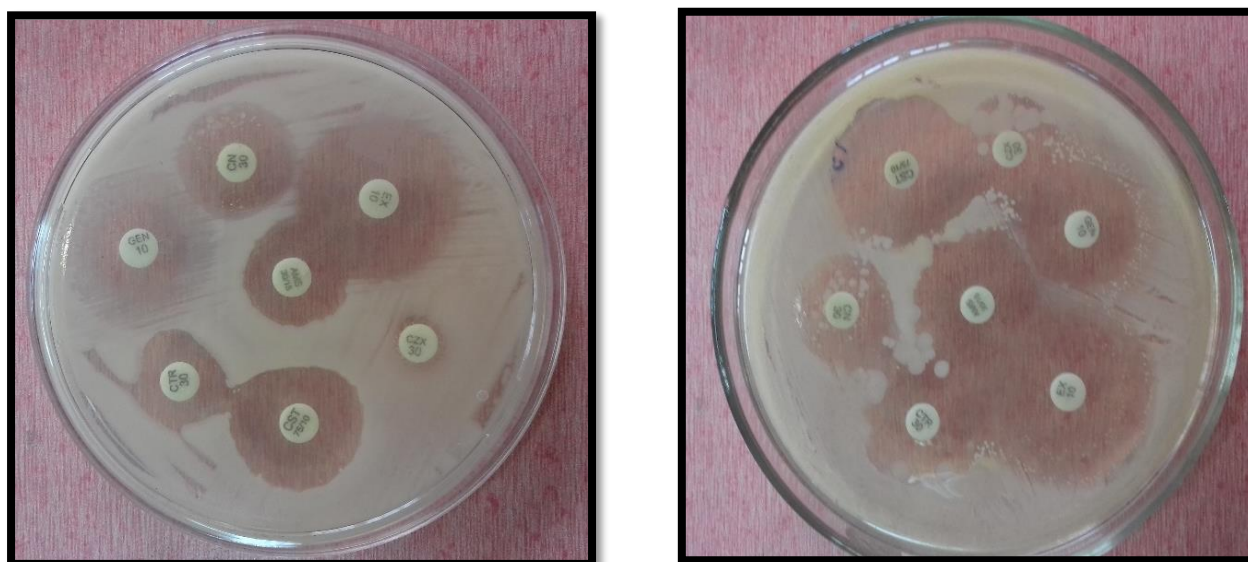


Figure 4 & 5: Displaying zone of inhibition pattern for different antibiotics

In the present study, the physical examination of milk revealed normal colour, consistency and odour. These observations were in accordance with Saravanan (1997) who observed that the colour, consistency and odour of milk from cattle affected with sub clinical mastitis were normal.

The animals affected with subclinical mastitis in the study did not exhibit any clinical signs. Schalm *et al.*, (1971), Radostits *et al.*, (2007) and Chakrabarti (2004) stated that there were no clinical signs associated with subclinical mastitis as well as no physical abnormalities in the milk. Similar findings were noticed in the present study.

Out of 46 dairy cattle examined, 15 (32.6 per cent) animals had single quarter affected and eight (17.3 per cent) animals had more than one quarter affected with sub clinical mastitis. The cumulative prevalence was 50 per cent. Among 167 quarters screened, 42 (25.1 per cent) quarters were found positive for Subclinical mastitis. Joshi and Gokhale (2004) reported that incidence of subclinical mastitis varied from 10 to 50 per cent in cows and 5 to 20 per cent in buffaloes in improved and peri urban dairy farms in India. Ahlner and Axelsson (2002) reported that the higher prevalence of subclinical mastitis was found 42.2 and 21.8 per cent of cow and quarter basis respectively.

Sharma *et al.*, (2008) reported that SCC was the most accurate test for the diagnosis of subclinical mastitis followed by Modified California Mastitis Test (MCMT) and Modified White Side Test (MWST). In the present study, estimation of somatic cells was done by using TANUCHEK SCC kit that detects increase in SCC precisely and was found very sensitive, specific as per the earlier studies of Yasothai (2017) and Nithya *et al.*, (2017).

Further in the present investigation antibiogram studies were also conducted for the positive milk samples for sub clinical mastitis by using seven antibiotics which were used frequently in the field for the treatment of mastitis and enrofloxacin was found to be more effective antibiotic among all the tested antibiotics in the present study followed by gentamicin, amoxicillin/ sulbactam, cefalexin, ceftriaxone, cefoperazone/ tazobactam and least was ceftizoxime.

The susceptibility patterns in the present study was comparable to the existing reports by Revathi *et al.*, (2016), Dhakal *et al.*, (2007), Kumar and Sharma (2002) have found that highest sensitivity were shown to enrofloxacin and gentamicin. Similar antibiogram patterns were reported by Sumathi *et al.*, (2008) and Choudhuri (2000) and they suggested that enrofloxacin, gentamicin, amoxicillin/ sulbactam are not commonly used for treatment of mastitis resulting in the higher sensitivity of these drugs.

Conclusion

In the present study, out of 167 quarters tested, only 42 samples (25.15 per cent) were found positive for subclinical mastitis. None of the milk samples were found as clinical cases. In the current study, TANUCHEK- SCC test kit was used to estimate somatic cell count to detect sub clinical mastitis cases which in turn gives picture of udder health status in dairy cows. Upon antibiogram studies of subclinical samples, enrofloxacin was found most sensitive antibiotic. Hence, prompt screening of animals for increase in somatic cell count and antibiogram studies are required regularly to select appropriate antibiotic for treatment purpose and also to reduce the risk of development of multi drug resistance which is one of the biggest threat to human and animal health.

Conflict of Interests

There is no conflict of interest.

Publisher Disclaimer

IJLR remains neutral concerning jurisdictional claims in published institutional affiliation.

References

1. Ahlner, S. and Axelsson, A. (2002). Analysis of bacterial growth and the prevalence of sub-clinical mastitis from bulk tank samples and individual samples from dairy herds in a region in Uruguay. *Minor Field Studies International Office, Swedish University of Agricultural Sciences.* (214), 16- 24.
2. Bachaya, H.A., Raza, M.A., Murtaza, S. and Akbar, I.U.R. (2011). Subclinical bovine mastitis in Muzaffargarh district of Punjab (Pakistan). *Journal of Animal and Plant Sciences.* (21), 16–19.
3. Bauer, A.W., Kirby, W.M.M., Sharrin, J. C. and Turk, M. (1966). Antibiotic susceptibility testing using a standard single disc method. *American Journal of Clinical Pathology*, (45), 493-496.
4. Chakrabarti, A. (2004). *A textbook of Preventive Veterinary Medicine, 3th Ed.* Kalyani Publication, New Delhi. 513-516.
5. Choudhuri, P.C. (2000). Status paper on mastitis in Andhra Pradesh. Proceedings of round table conference (IAAVR) on mastitis. Feb. 18-19, Izatnagar, 84-92.
6. Dhakal, I.P., Dhakal, P. Koshihara T. and Nagahata, H. (2007). Epidemiological and bacteriological survey of buffalo mastitis in Nepal. *Journal of Veterinary Medical Science*, (69), 1241-1245.
7. Durr, J.W., Cue, R.I., Monardes, H.G., Moro-Mendez, J, and Wade, K.M. (2008). Milk Losses Associated with Somatic Cell Counts per Breed, Parity and Stage of Lactation in Canadian Dairy Cattle. *Livestock Science*, (117), 225-232.
8. Giannechini, R., Concha, C., Rivero, R., Delucci, I. and Moreno Lopez, J. (2002). Occurrence of clinical and sub-clinical mastitis in dairy herds in the west littoral region in Uruguay. *Acta Veterinaria Scandinavica*, (43), 221–230.
9. Joshi, S and Gokhale, S. (2004). Status of mastitis as an emerging disease in improved and periurban dairy

- farms in India. 23rd World Buiatrics Congress Quebec City, Canada.
10. Kumar, R. and Sharma, A. (2002). Prevalence, etiology and antibiogram of mastitis in cows and buffaloes in Hisar, Haryana. *Indian Journal of Animal Sciences*, (72), 361-363.
 11. Nithya, P., Sivakumar, K., Aruljothi, A. and Tirumurugaan, K.G. (2017). Comparison of TANUCHEK Somatic Cell Count Test and CMT for the Detection of Sub - Clinical Mastitis in the Milk of Lactating Dairy Cows of Coimbatore District. *International Journal of Livestock Research*, 7(8), 173-178.
 12. Radostits, O.M., Gay C.C., Hinchcliff, K.W. and Constable, P.D. (2007). *Veterinary Medicine. A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 10th ed.*, Saunders Elsevier, Spain.
 13. Revathi, P., Rani prameela, D., Syaama sundar, N. and Deepika kumari, G. (2016). Antibiogram of Bovine Mastitis in and around Proddatur, District Kadapa, (A.P.). *The blue cross book*, (33), 65-66.
 14. Rodriguez, S. I., Gianola, D and Shookg, E. (2000). Evaluation of Models for Somatic Cell Score Lactation Patterns in Holsteins, *Livestock Production Science*, (67), 19-30.
 15. Saravanan, P. (1997). *A comparative study on the efficacy of diagnostic tests in the diagnosis of bovine subclinical mastitis*. (M.V.Sc., Thesis), Tamil Nadu Veterinary and Animal Science University, Chennai, India.
 16. Schalm, O.W., Carroll, E. J. and Jain, N. C. (1971). *Bovine Mastitis*. Lea and Febiger, Philadelphia. 102-103.
 17. Sharma, N., Maiti, S.K. and Pandey, V. (2008). Sensitivity of indirect tests in the detection of subclinical mastitis in buffaloes. *Veterinary Practitioner*, (9), 29–31.
 18. Stefanakis, A., Boscov, C., Alexopoulos, C. & Samartzi, F. (1995). Frequency of subclinical mastitis and observations on somatic cell counts in ewes' milk northern Greece. *Animal Science*, (61), 69.
 19. Sumathi, B.R., Veeregowda, B.M. and Amitha gomes, R. (2008). Prevalence and antimicrobial profile of bacterial isolates from clinical bovine mastitis. *Veterinary world*. (1), 237-238.
 20. World Health Organization. (2000). Overcoming antimicrobial resistance. WHO Report on Infectious Diseases. Retrieved from <http://www.who.int/infectious-diseasereport/2000/index.html>
 21. Yasothai, R. (2017). Comparison of Diagnostic Tests for the Detection of Sub-Clinical Mastitis in Dairy Farms of Erode District. *International Journal of Science, Environment and Technology*, 6 (2), 1321 – 1326.
